

***Ascobolus stictoideus* Speg. AEB 1317 (= PDD 117248)**

Collection site: Lower Hutt, New Zealand. Along the Waiwhetu Stream, in grassy areas that border Callaghan Innovation (base of the Wainuiomata Hill Road)

Collection date: 12 November 2017

Substrate: European rabbit (*Oryctolagus cuniculus*) dung; **Collector & Identifier:** Dan Mahoney

Voucher materials: no dried herbarium material but two Shear's mounting fluid (SMF) semi-permanent slide mounts; several in-situ dissecting scope photos of apothecia on the dung and a number of photos of microscopic detail from water, SMF and Melzer's reagent slide mounts.

Other fungi also present on rabbit pellets from this collection include the following: *Ascobolus minutus*, *Collariella bostrychodes* (\equiv *Chaetomium bostrychodes*), *Delitschia* (*patigonica*?), *Iodophanus carneus*, *Mucor* spp., *Pilaira anomala*, *Podospora curvicolla*, *Saccobolus caesariatus*, *Sporormiella australis*

Dan's brief comments & description: Apothecia seen beneath a tangle of *Pilaira* and *Mucor* overgrowth on several rabbit pellets. Perhaps more numerous but I had to focus on those more obvious apothecia with protruding asci. In-situ and microscopic-detail photos were taken on the 21st and 23rd of November. All photos of microscopic detail in this pdf were from water mounts.

Apothecium excipulum a rather large-celled textura angularis. **Paraphyses** numerous, filamentous, uniform throughout (not apically specialized), hyaline, septate. **Asci** clavate to saccate with some faint ascus wall bluing but not the 'deep blue' of Brummelin's description, variable in size – a more typical one measuring $202.5 \times 45 \mu\text{m}$. **Ascospores** $28\text{--}31 \times 15\text{--}19 \mu\text{m}$, ellipsoid, distinctly punctate (warty to irregular warty), brownish (violet brown to violet to purplish when fresh and mature, or nearly mature, inside asci), also with a one-sided gelatinous sheath Overall, a reasonably good match to Brummelin's description – see that description and the illustrations on the next two pages.

There is one PDD collection of *Ascobolus stictoideus* on the Systematics Collections Data website as of December, 2020 (excluding the present collection): PDD 76919 (= AEB 697) identified by Ann Bell from wombat dung in Australia.

Brummelen J. Van. 1967. A world monograph of the genera *Ascobolus* and *Saccobolus* (Ascomycetes, Pezizales). Persoonia, supplement Vol. I. Rijksherbarium, Leiden. Pages 76–78.

3. ASCOBOLUS STICTOIDEUS Speg.—Fig. 11; Pl. 2, figs. G, H

Ascobolus stictoides Speg. in *Michelia* 1: 474. 1879. — *Ascobolus brunneus* var. *stictoides* (Speg.) Heimerl in *Jber. kk. Ober-Realschule Bez. Sechshaus Wien* 15: 14. 1889. — Holotype: LPS 26119.

Apothecia scattered, gregarious or closely crowded, completely immersed or rarely superficial, 350–600 μ diameter, 400–650 μ high. Receptacle at first closed and globular or pyriform, then opening by irregular rupturing of the wall, watery-white, greenish-yellow or olive in colour; thinly tomentose or rarely smooth; without a margin. Disk flat or slightly concave, pale olivaceous to almost colourless, with a few almost black, ripe asci strongly protruding. Hymenium 230–280 μ thick, containing up to 40 asci. Hypothecium very thin, of isodiametric cells 6–12 μ diameter. Flesh not sharply differentiated or very thin. Excipulum about 15 μ thick, of more or less isodiametric, polygonal, thick-walled cells 9–25 μ diameter (textura angularis), with pale yellowish cell-walls, often covered with a thin layer of interwoven, cylindrical, irregularly branched, hyaline, 4–9 μ wide hyphae. Asci clavate or clavate-saccate, gradually tapering downwards into a rather thick base, rounded above, 150–400 \times 39–55 μ , 8-spored; the wall deep blue in Melzer's reagent. Ascospores biseriate or irregularly disposed, ellipsoid; at first hyaline, then pale violet, finally dark violet, (25.5–)26.5–30.5(–32) \times (14.5–)16–17.5 μ , ornamented with rather coarse, rounded warts; pigment often rather thick up to 1.5 μ ; with

unilateral, mucilaginous substance. Paraphyses filiform, simple, septate, 2.8–3.8 μ thick, not enlarged upwards, sometimes slightly narrowed at the tip 2.5–3.2 μ thick, hyaline, embedded in pale yellowish or colourless mucus.

On dung of cow, horse, sheep, dog, rabbit, muskrat, and goose, also on soil dirtied with dung.

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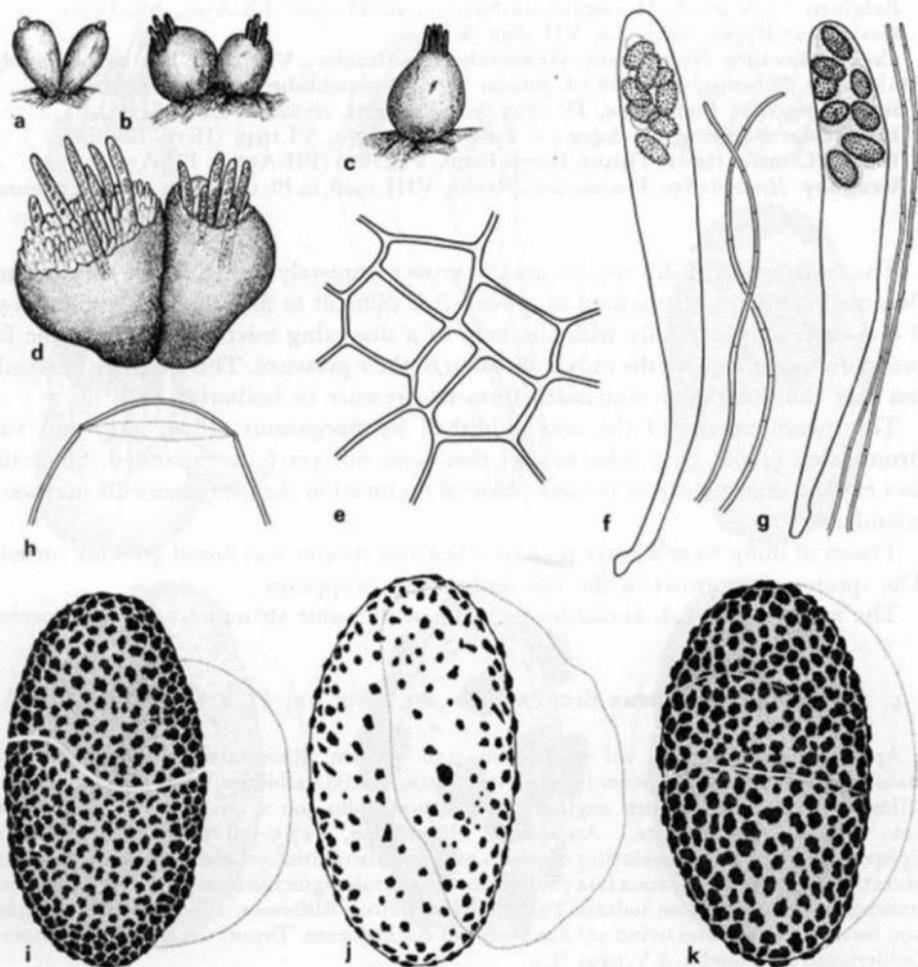


Fig. 11. — *Ascobolus stictoides*: a-c, habit of fruit-bodies $\times 30$; d, two fruit-bodies $\times 65$; e, texture of excipulum seen from outside $\times 740$; f, g, asci and paraphyses $\times 175$; h, top of ascus $\times 1000$; i-k, ascospores $\times 1600$. (From living material.)

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On dung of cow, horse, sheep, dog, rabbit, muskrat, and goose, also on soil dirtied with dung.

ETYMOLOGY.—From Greek, *στεικτος*, punctured and *ειδος*, like.

ILLUSTRATION.—van Brummelen in *Persoonia* 2: 196 f. 2. 1962.

SPECIMENS EXAMINED.—**Netherlands**: van Brummelen, Pinetum, Putten, 3.V.1958 (slide, L); van Brummelen 59.3, Vierhouten, 20.IV.1959 (slide, L).

Belgium: Culot 2348, Montignies-sur-Sambre, 2.XII.1926 (BR-A305, BR-A358).

Germany: Wagner, Schmilka, VII.1892 (S-A626).

Czechoslovakia: Petrak, Mähr.-Weisskirchen (= Hranice), VI.1924 (ZT-A104); Velenovský, Mnichovice, Bohemia, 1.V.1928 (*A. pallidus* Vel., an unpublished name, PR 150266).

Italy: Spegazzini, Conegliano, IV.1879 (holotype of *A. stictoides*, LPS 26119).

Indonesia: Boedijn 4934, Bogor (= Buitenzorg), Java, VI.1949 (Herb. Boedijn).

U.S.A.: Connecticut: Thaxter, Green Farm, VII.1890 (FH-A3109, FH-A3111).

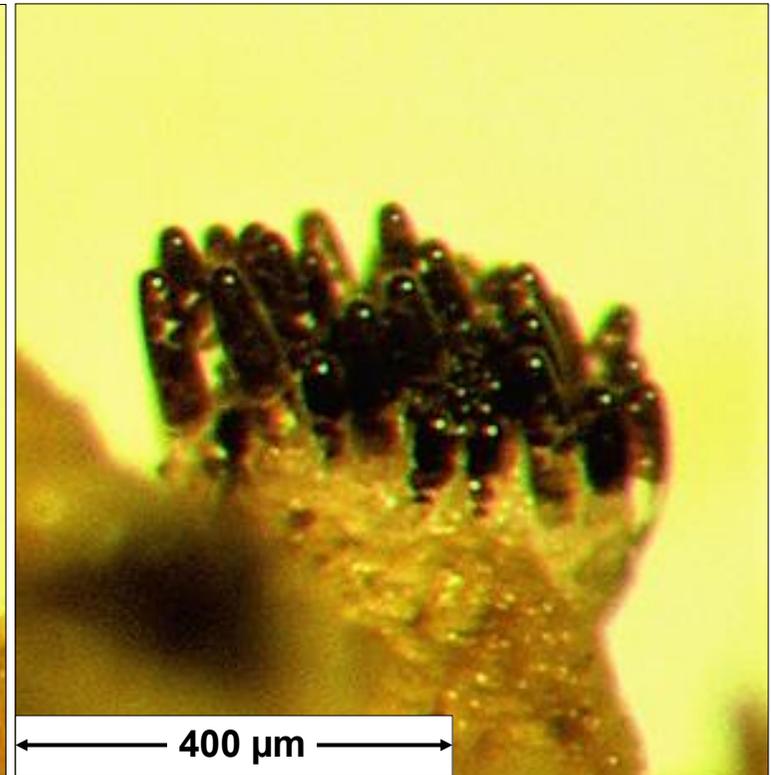
Uruguay: Herter 83630, Rocha, dept. Rocha, VIII.1928, in Pl. urug. exs. 1222 (*A. brunneus*, M).

The fruit-bodies of this species usually grow completely buried in the substratum. Because of this cryptic method of growth it is difficult to find the fruit-bodies, even if one looks very carefully with the help of a dissecting microscope. Often the far protruding ripe asci are the only indication of their presence. This species is certainly less rare than might be concluded from its presence in herbaria.

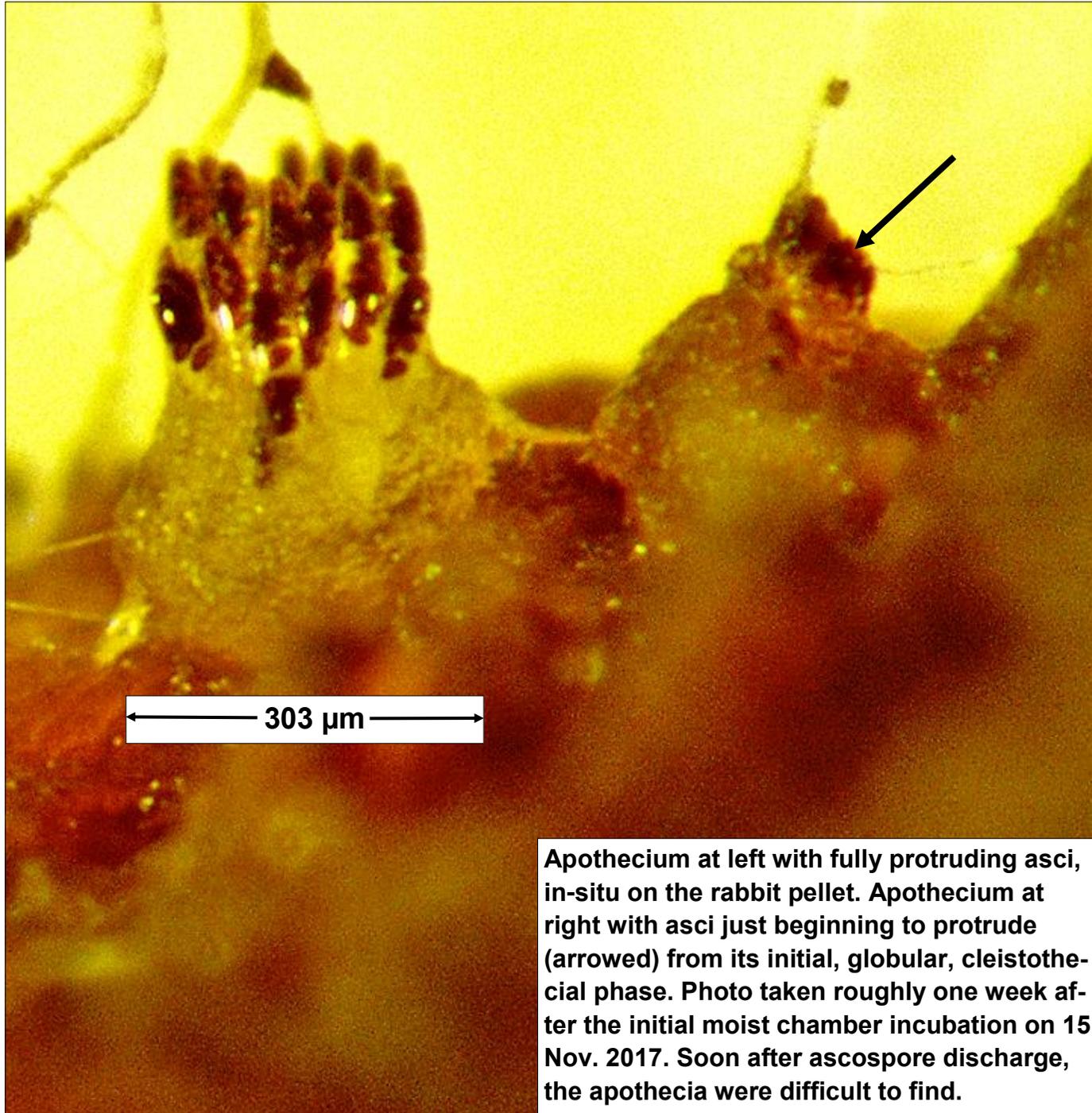
The measurements of the asci published by Spegazzini (1879: 474) and van Brummelen (1962: 197) refer to asci that were not yet fully expanded. Since the asci expand enormously in the last phase of maturation the measurements may vary considerably.

Traces of dung were always present when this species was found growing on soil. The species often grows in the soil underneath droppings.

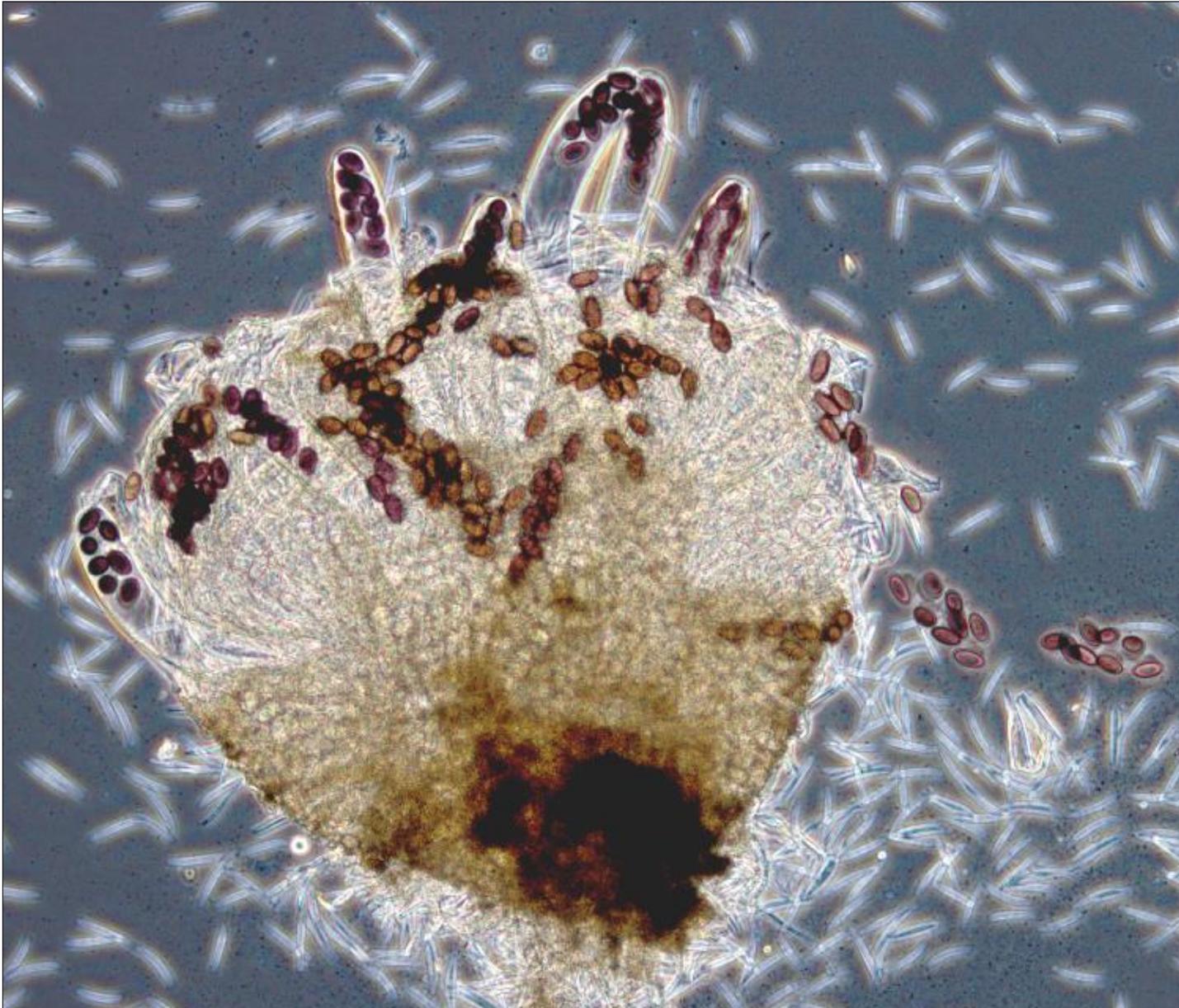
The episporium of *A. bistisii* seems to be of the same structure as in this species.



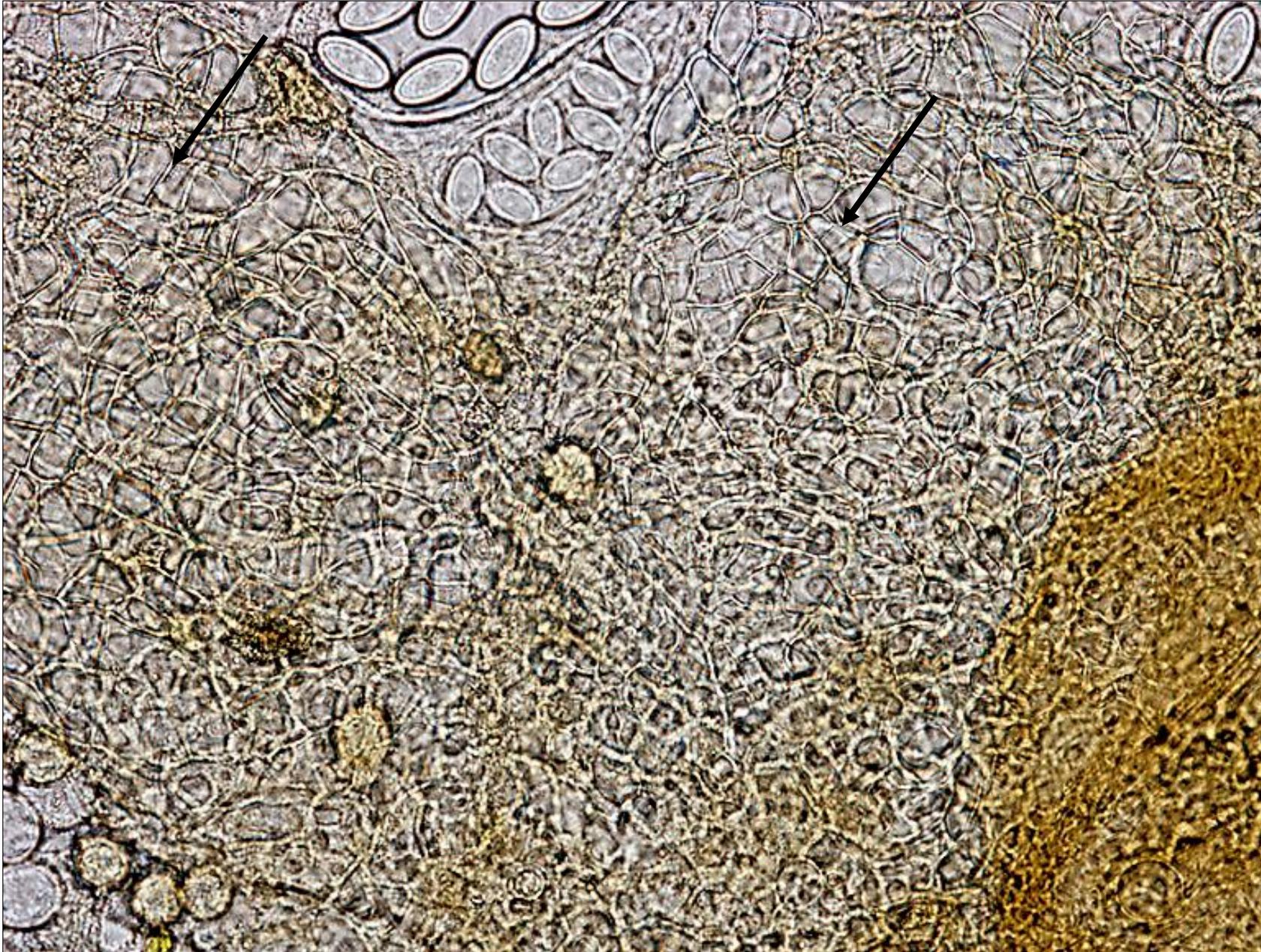
Apothecia with protruding asci, in-situ on the rabbit pellet. Photos taken roughly one week after the initial moist chamber incubation on 15 Nov. 2017. Soon after ascospore discharge, the apothecia were difficult to find. Note the globular cleistothecial beginnings of apothecia in the left photo (arrowed).



Apothecium at left with fully protruding asci, in-situ on the rabbit pellet. Apothecium at right with asci just beginning to protrude (arrowed) from its initial, globular, cleistothelial phase. Photo taken roughly one week after the initial moist chamber incubation on 15 Nov. 2017. Soon after ascospore discharge, the apothecia were difficult to find.



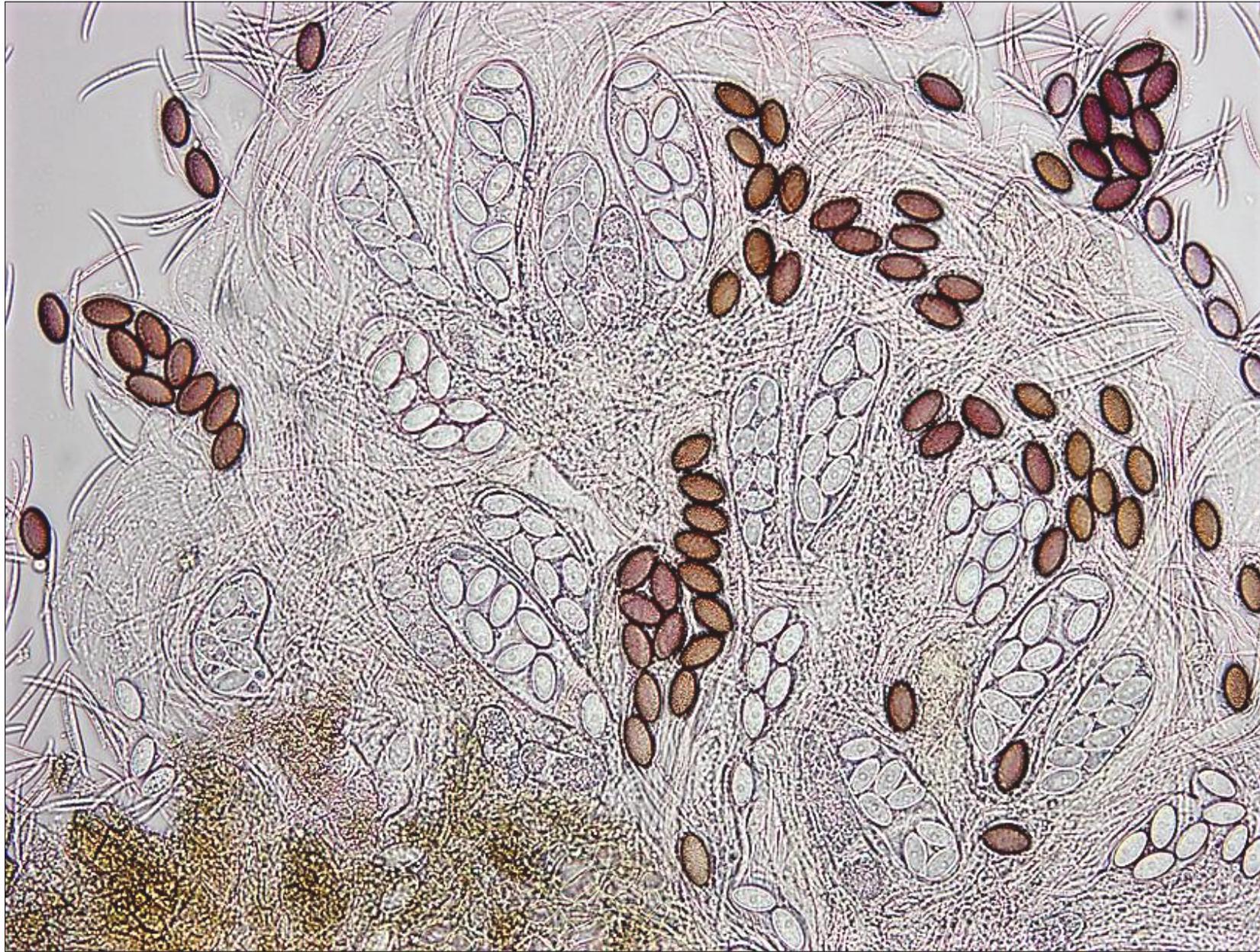
Whole, somewhat squashed, apothecium in a water mount, using a $\times 10$ objective and phase microscopy. Aside from the ellipsoidal brownish to eventually dark violet ascospores, there are scattered macroconidia of a *Fusarium* sp.



A portion of the apothecial excipulum showing its hyaline to faintly yellow textura angularis tissue. Water mount, using a $\times 40$ objective and brightfield microscopy.



Hymenial squash showing asci with ascospores in various stages of maturity – hyaline to lightly pigmented & faintly warty, brown & warty and finally dark violet & warty. Note the *Fusarium* macroconidia scattered throughout, particularly at the left and upper right. Water mount, using a $\times 20$ objective and phase microscopy. See a brightfield view of the same field on the next page.



Hymenial squash showing asci with ascospores in various stages of maturity – hyaline to very lightly pigmented & faintly warty, brown & warty and finally dark violet & warty. Note the *Fusarium* macroconidia scattered throughout, particularly at the left and upper right. Water mount, using a $\times 20$ objective and brightfield microscopy. See a phase view of the same field on the previous page.



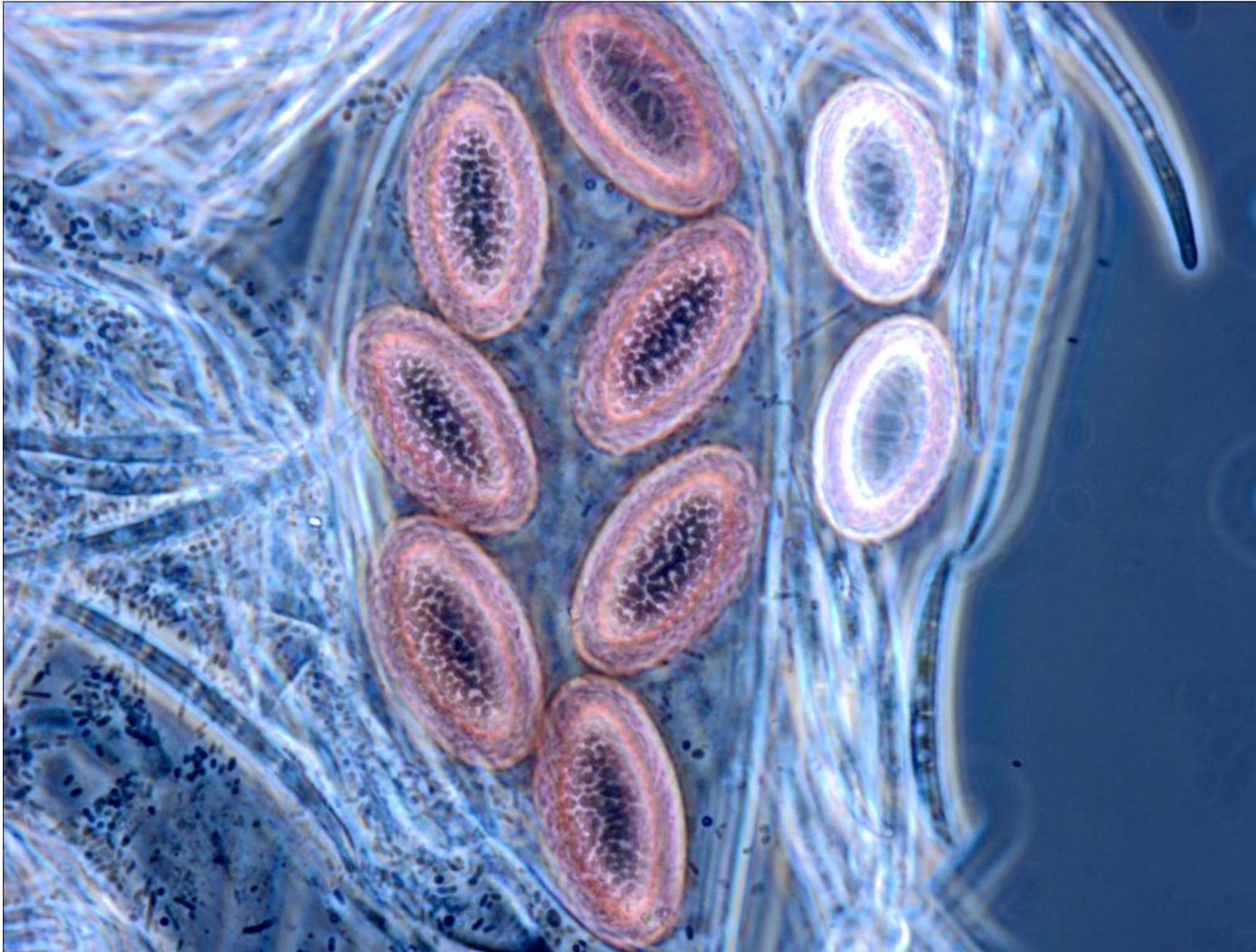
Hymenial squash showing asci with ellipsoidal ascospores in various positions within the asci and in various stages of maturity – hyaline to lightly pigmented & faintly warty and finally dark violet & warty. Note also the narrow hyaline paraphyses sandwiched among the asci (arrowed). Water mount, using a ×40 objective and brightfield microscopy.



Asci, ascospores and scattered *Fusarium* macroconidia. Left photo: Note the ellipsoidal ascospores in various stages of maturity – hyaline to light or darker brown & warty then light violet to finally dark violet and warty. Right photo: Fully mature ascus and ascospores. Water mount, using a $\times 40$ objective and brightfield microscopy.



Hymenial squash showing asci with ellipsoidal ascospores in various stages of maturity – hyaline to lightly pigmented & faintly warty, light violet & warty and finally dark violet & warty. Note also the narrow hyaline paraphyses sandwiched among the asci (arrowed). Water mount, using a $\times 40$ objective and brightfield microscopy.



59 × 3 μm



Left photo: Mature violet ascospores with focus on surface warts. Note also scattered *Fusarium* macroconidia. Right photo: a single enlarged *Fusarium* macroconidium. Both photos water mounts using ×100 objectives and phase microscopy.