

***Saccobolus citrinus* Boud. & Torrend 1911 – AEB 1361 (= PDD 120029)**

**Collected:** March 2023

**Substrate:** Toggenburg milking goat dung (The goats eat a varied diet of grass/herbage with gorse and mixed native vegetation which was cut and delivered to them. They receive very little or no chemical treatment.)

**Collection site:** NZTM grid ref. E1770110 N5447412 , a private residence surrounded by native bush off Moonshine Hill Road, Upper Hutt

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

**Voucher materials:** dried herbarium specimen including 3 goat pellets covered with numerous apothecia and accompanied by 4 Shear's mounting fluid (SMF) semi-permanent microscope slides; dissecting scope photos of fresh apothecia on the dung and compound scope microscopic views of paraphyses, asci and ascospore clusters in various stages of maturity; Dan's comments.

**References consulted for *Saccobolus citrinus*:** (listed in chronological order)

- 1) Brummelen J. (van). 1967. A World-monograph of the genera *Ascobolus* and *Saccobolus* (Ascomycetes, Pezizales). Persoonia, supplement, I: 1-260.
- 2) Bell A. 1983. Dung Fungi: An Illustrated Guide to Coprophilous Fungi in New Zealand. Victoria University Press, Wellington. 88 pages.
- 3) Doveri F. 2004. Fungi Fimicoli Italici. Vicenza, A.M.B.-Fondazione Centro Studi Micologici, 1104 p., 158 pl. **Includes a key to *Saccobolus* and a description with illustrations of *S. citrinus*.**
- 4) Bell A. 2005. An Illustrated Guide to the Coprophilous Ascomycetes of Australia. CBS Biodiversity Series No. 3, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, 172 pages.
- 5) Mungai P.G. et al. 2012. Coprophilous ascomycetes in Kenya – *Saccobolus* species from wildlife dung. Mycosphere 3(2), 111–129. **For their description of *S. citrinus* with illustrations see the next page.**

Mungai P.G., Chukeatirote E., Njogu J.G. & Hyde K.D. 2012. Coprophilous ascomycetes in Kenya – *Saccobolus* species from wildlife dung. *Mycosphere* 3(2), 111–129. (Figs.2A–I & 3A–F)

Portions of p. 113 are reproduced below with Figs. 2 & 3 from pages 114 & 115 on the next page.

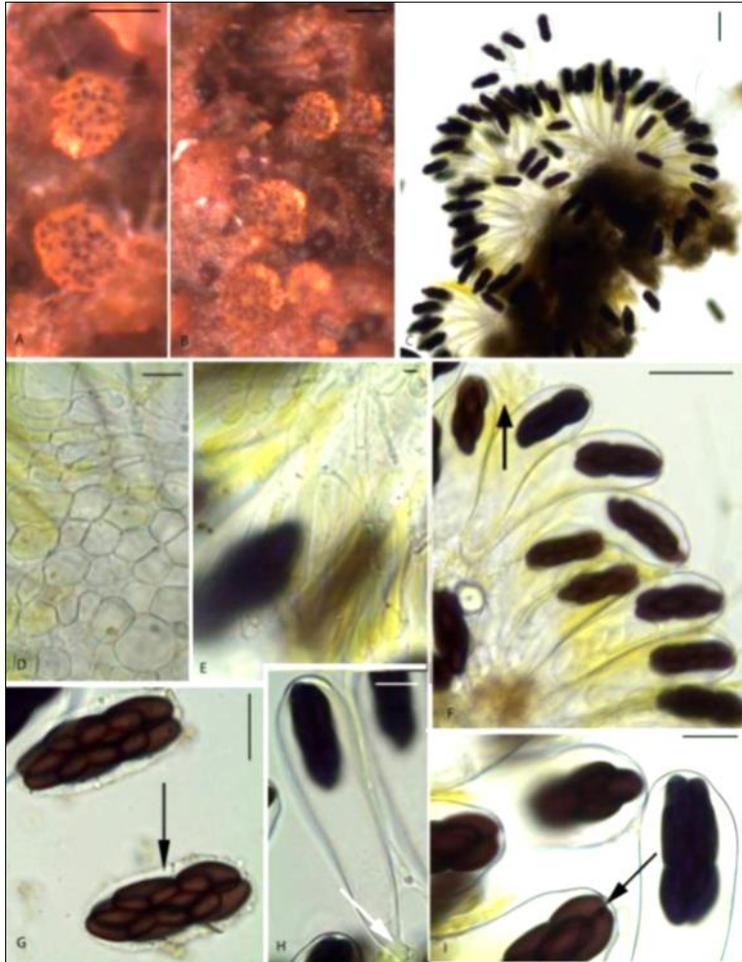
“*Saccobolus citrinus* Boud. & Torrend, Bull. Soc. Mycol. Fr. 27: 131, 1911.

**Ascomata** apothecioid, scattered or gregarious, superficial, sessile, 180–215 µm high, 205–315 µm diam. Receptacle outer surface bright to lemon yellow, smooth, without margin, subglobose, pulvinate or lenticular at maturity. Disc convex, membranaceous, lemon yellow, dotted with blackish tips of ripe protruding asci. Hypothecium and medullary excipulum not differentiated from ectal excipulum. Ectal excipulum thin composed of textura globulosa, pale yellow to yellowish grey cells, 8–20 × 8–18 µm. **Paraphyses** cylindric-filiform, simple, septate, exceeding asci, 3 µm broad, not branched, inflated tips with abundant yellow pigmentation. **Asci** 113–148 × 27–34 µm, 8-spored, unitunicate, broadly clavate, thick-walled, flat apex, walls turning blue in Melzer’s reagent; stipe short, 8–10.5 × 5.5–6 µm, operculate. **Ascospores** 17–21 × 8–9 µm, arranged according to van Brummelen pattern I, ellipsoidal-fusoid, violet to brownish purple, slightly asymmetrical, with truncate or blunt ends, verruculose, sometimes with fissures, thick-walled; clusters elongated, 45–50.5 × 15–20 µm, compact and firmly enclosed all round in a narrow gelatinous envelope 2–4 µm thick.

**Material examined (4 isolates)** – KENYA, Nairobi National Park, Nairobi Province, GPS 37M0255729 9849626, altitude 1680m, impala dung, 20 August 2010, Paul Mungai, KWSNNP020-2010; Nairobi National Park, Nairobi Province, GPS 37M0255191 9849808, altitude 1693m, wooded grassland, Cape buffalo dung, 20 August 2010, Paul Mungai, KWSNNP015-2010; Nairobi National Park, Nairobi Province, GPS 37M0257082 9850692, altitude 1668m, wooded grassland, giraffe dung, 20 August 2010, Paul Mungai, KWSNNP017B-2010; Nairobi National Park, Nairobi Province, GPS 37M0254965 9850592, altitude 1685m, wooded grassland, hippopotamus dung, 20 August 2010, Paul Mungai, KWSNNP021-2010.

**Notes** – *Saccobolus citrinus* Sect. *Saccobolus* Boud. is similar to *S. succineus* Brumm., (van Brummelen 1969, Doveri 2004). However, it can be distinguished from these species by having lemon-yellow apothecia and notably truncate ended ascospores. In addition, the ascospores of *S. citrinus* (21–24.5 × 8–9.5 µm in this examination) are narrower and more finely warted (van Brummelen 1967, Doveri 2004, Bell 2005) while those of *S. succineus* are larger. *S. citrinus* is quite a common species on wildlife herbivore dung in Kenya and it is a new record for Kenya.”

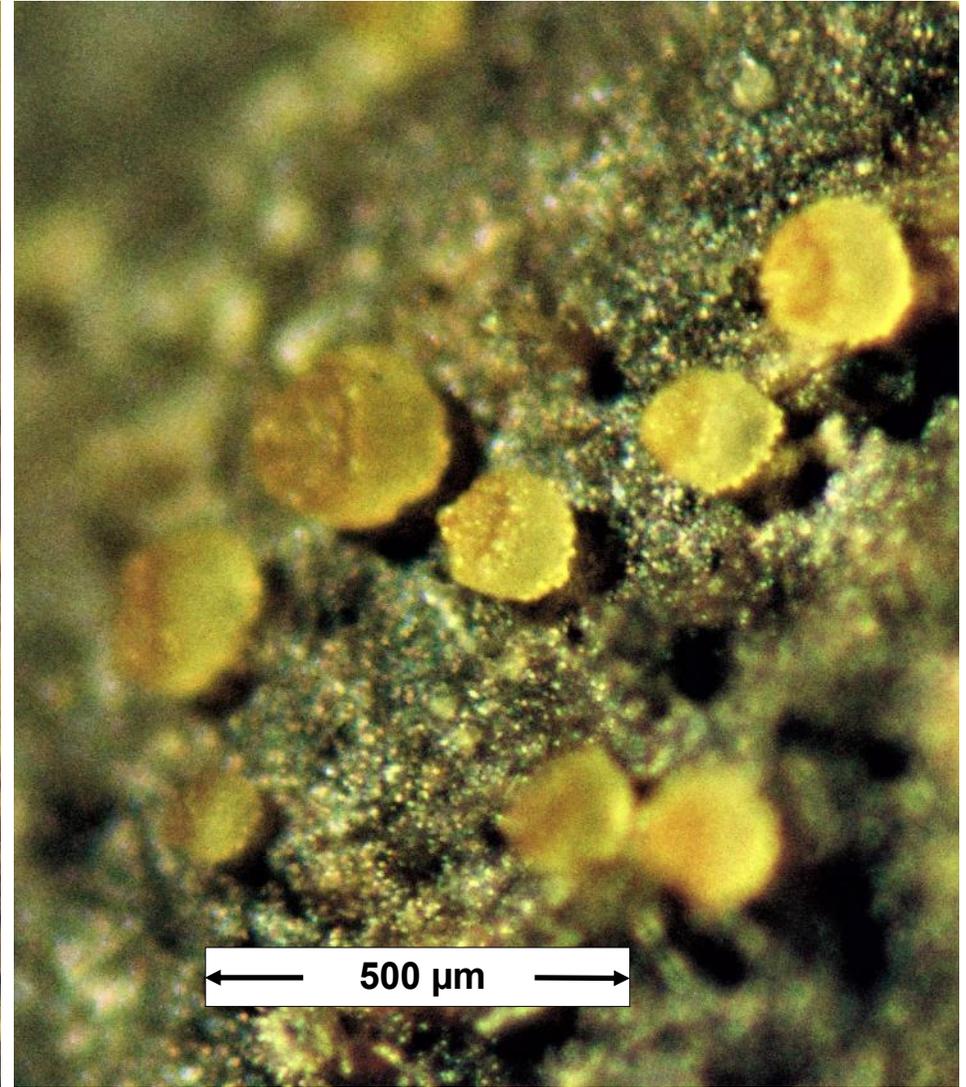
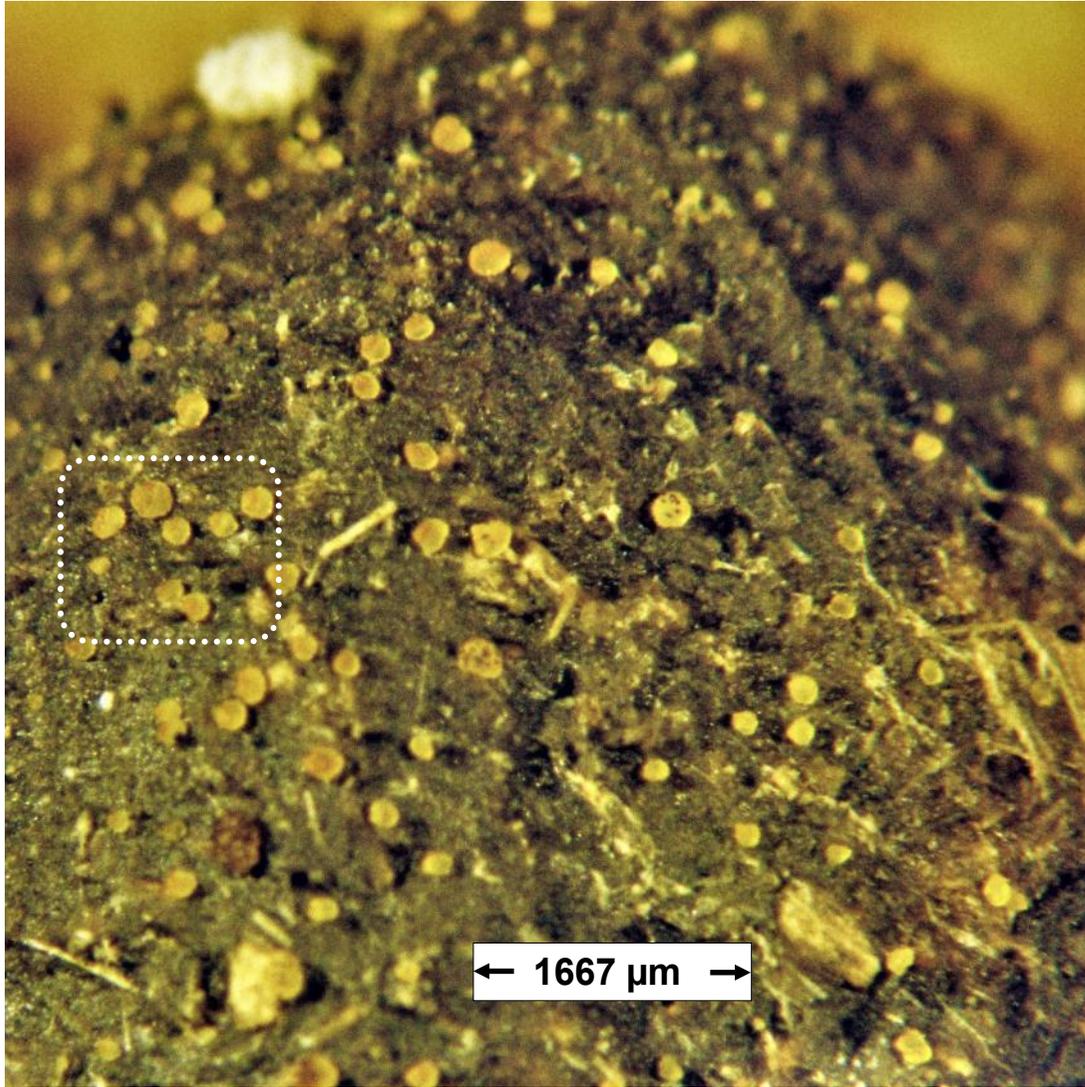
**Figs.2A-I & 3A-F**



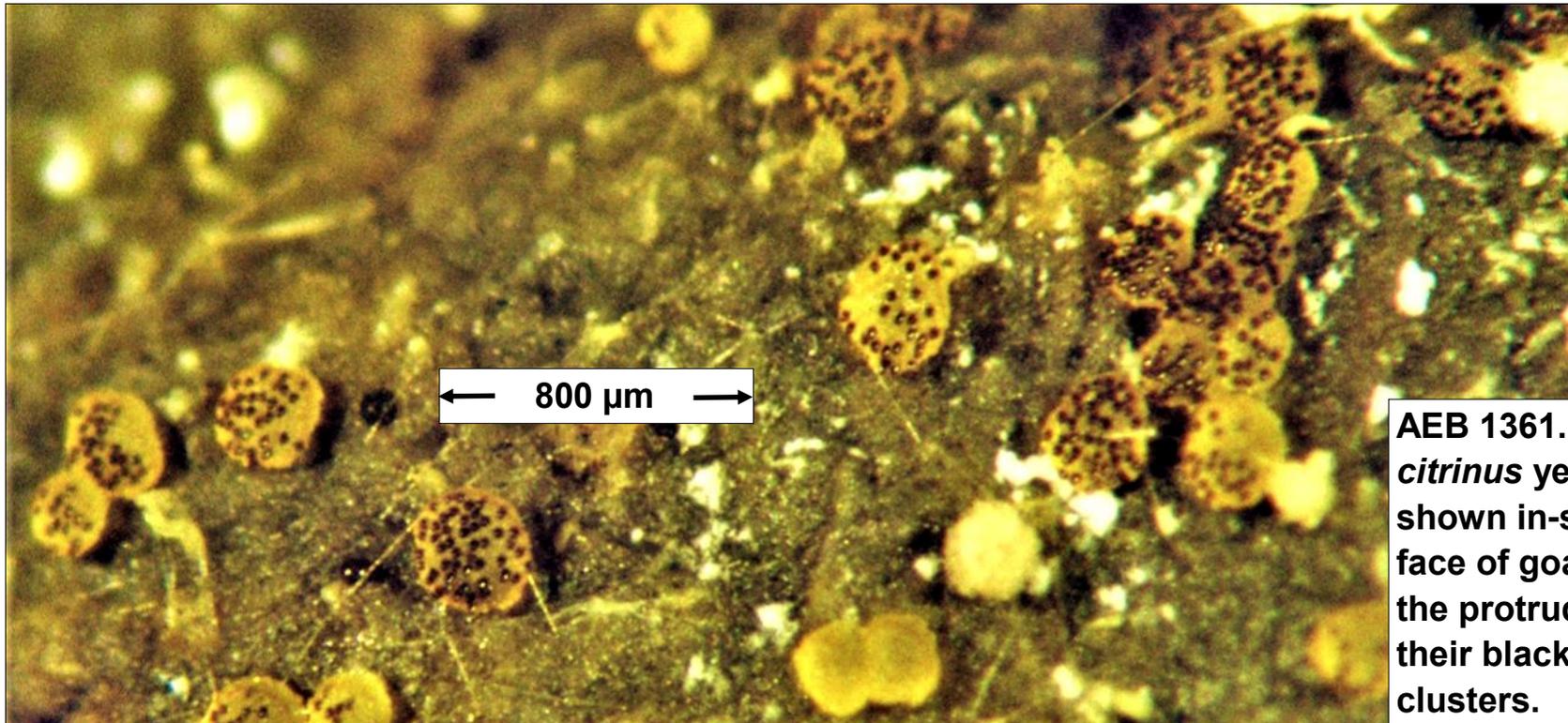
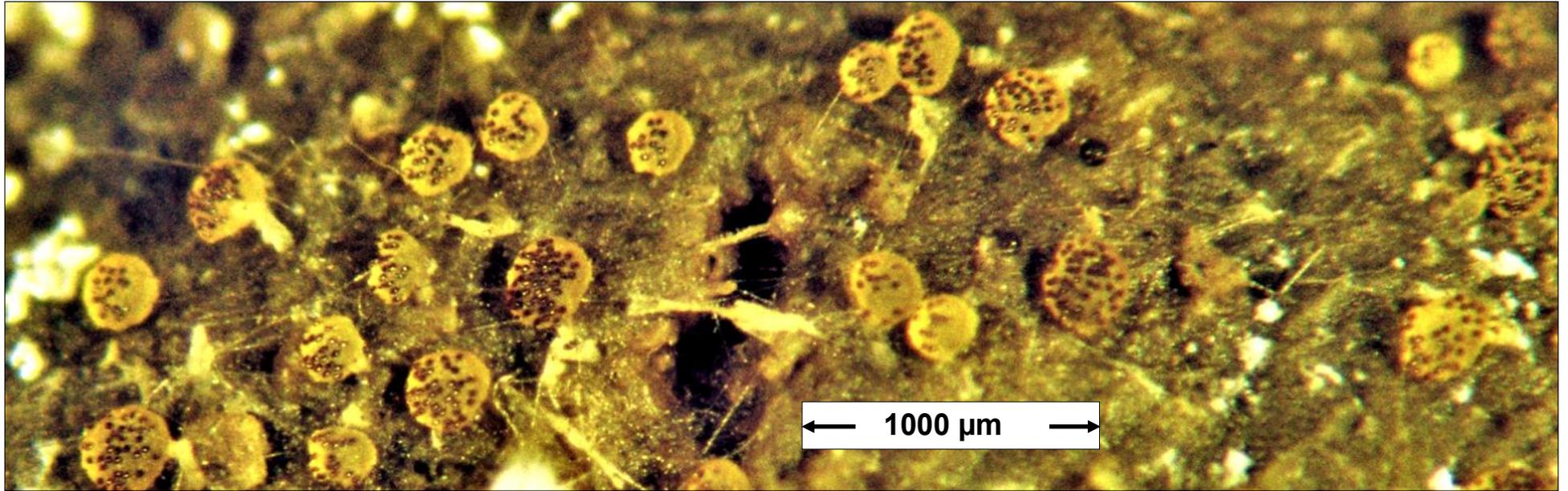
**Fig. 2** – *Saccobolus citrinus* (KWSNNP020-2010). **A-B** Ascata on dung. **C** Squashed ascoma. **D** Details of ectal excipulum near the margin. **E** Paraphyses filled with yellow pigment. **F** Mature asci and ascospores, note yellow mucus (arrow). **G** Mature ascospore clusters, note gelatinous sheath (arrow). **H** Mature ascus showing a stipe (white arrow) and an apex. **I** Apical portion of asci, note closed operculum (arrow). Scale bars: A-B = 500  $\mu$ m, C = 200  $\mu$ m, D = 20  $\mu$ m, E = 50  $\mu$ m, F = 20  $\mu$ m, G = 20  $\mu$ m, H = 20  $\mu$ m, I = 20  $\mu$ m.



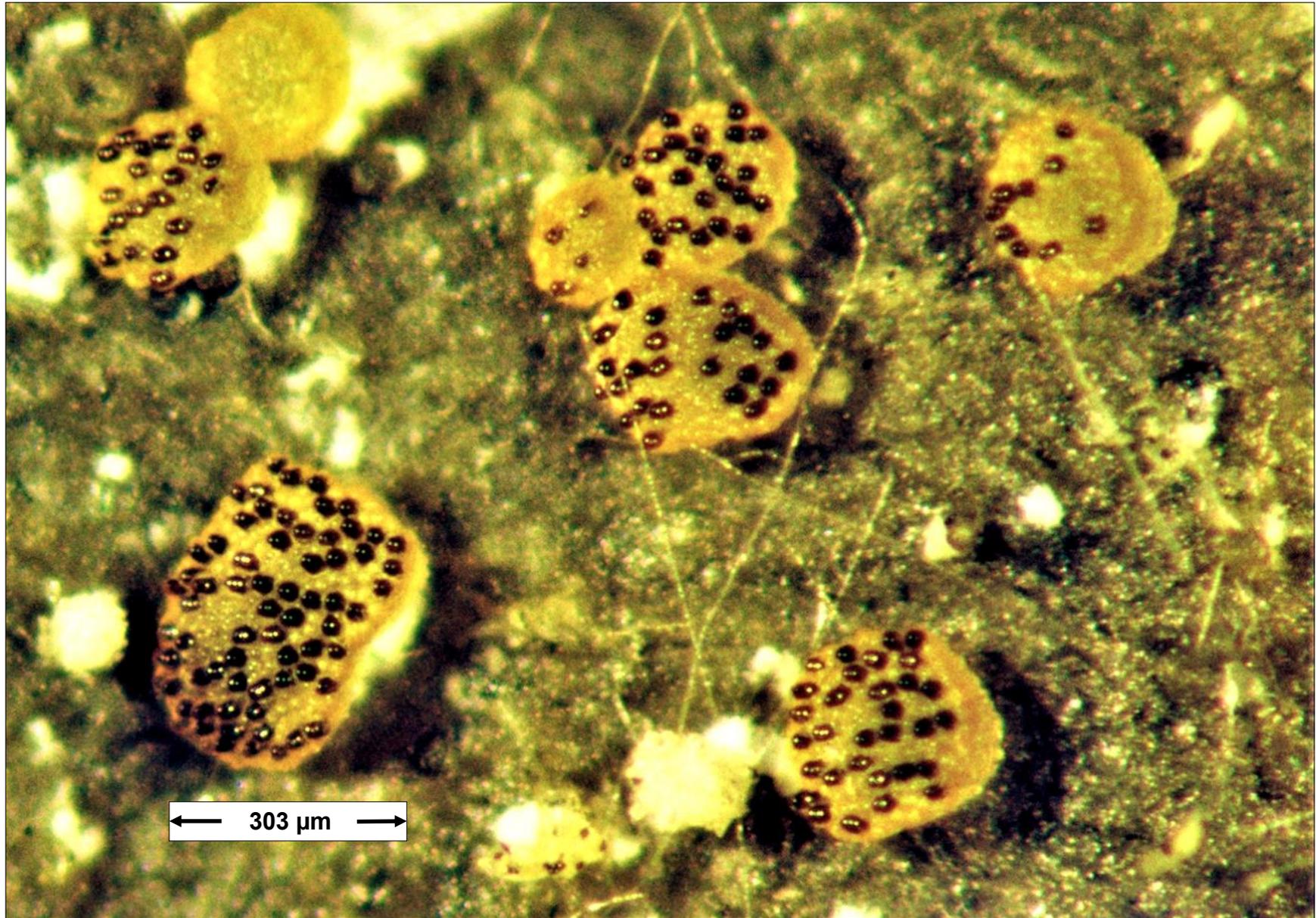
**Fig. 3** – *Saccobolus citrinus* (KWSNNP020-2010). **A** Ascus, note stipe (arrow). **B** Ascospore cluster, note gelatinous sheath (arrow). **C** Free ascospore cluster and an immature ascus, note spore arrangement (black arrow), operculum, apical ring (white arrow). **D-F** Features of asci, ascospores and paraphyses, note yellow mucus (black arrow) and verruculose episporium (white arrows). Scale bars: A-D = 20  $\mu$ m, E-F = 50  $\mu$ m.



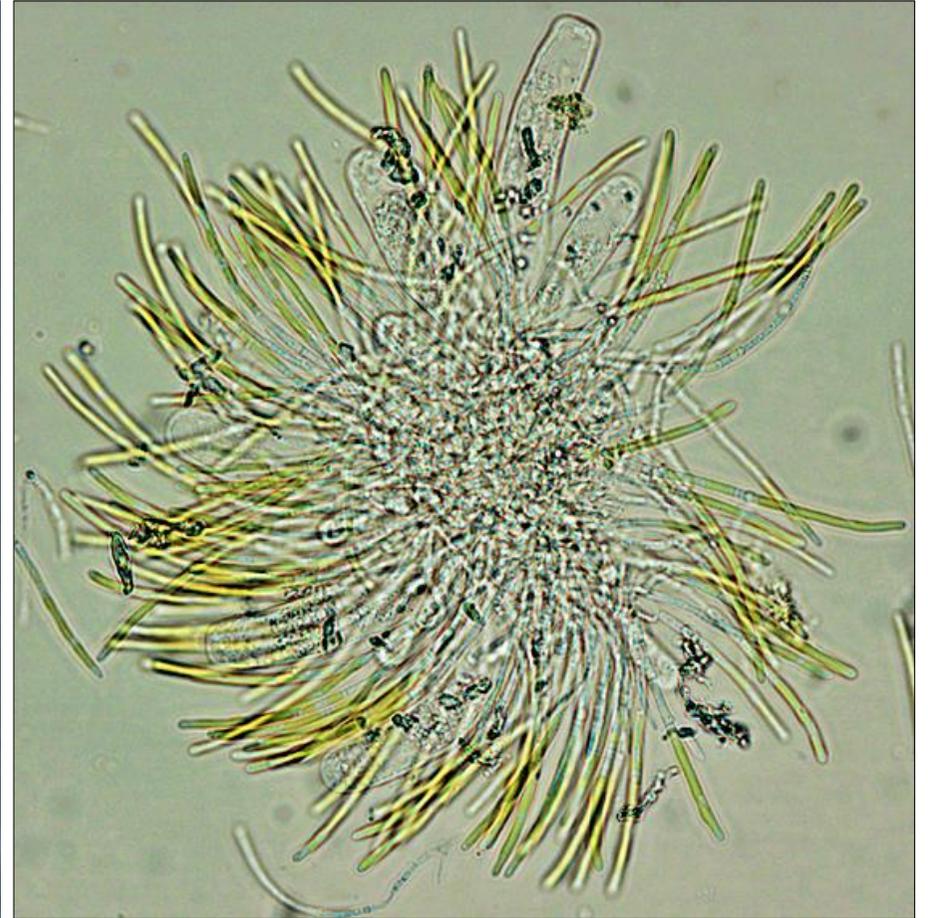
**AEB 1361. *Saccobolus citrinus* yellow apothecia shown in-situ on the surface of goat dung. Many apothecia here are young & without pigmented ascospore clusters. Such apothecia are shown in the dotted rectangle in the left-side photo. These are shown more highly magnified in the right-side photo.**



**AEB 1361. *Saccobolus citrinus* yellow apothecia shown in-situ on the surface of goat dung. Note the protruding asci with their blackish ascospore clusters.**



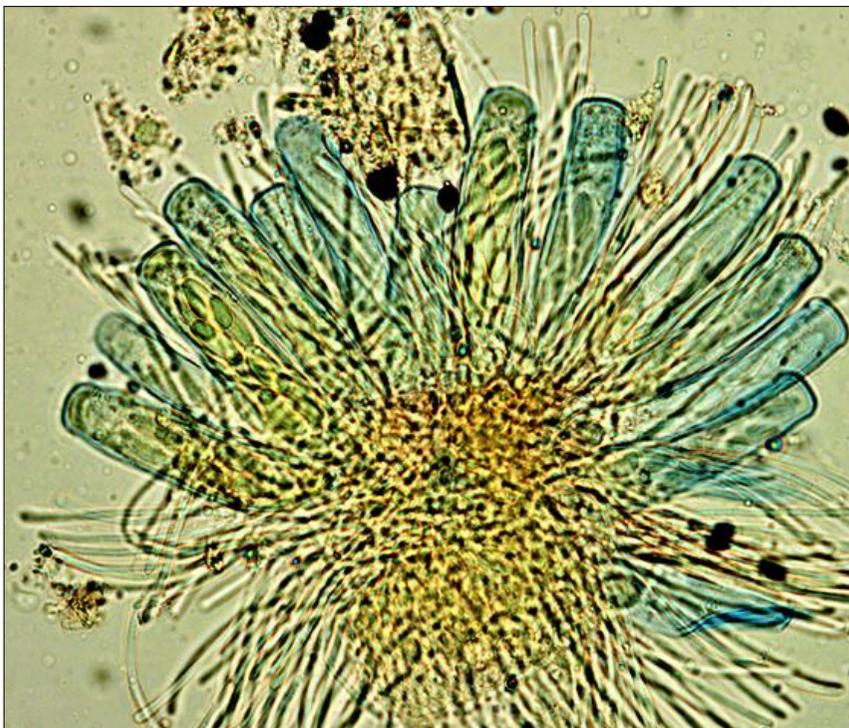
AEB 1361. *Saccobolus citrinus* yellow apothecia shown in-situ on the surface of goat dung. Note the protruding asci with their blackish ascospore clusters.



**AEB 1361. Immature *Saccobolus citrinus* with young asci and unbranched yellow, septate paraphyses. Both photos from water mounts. Left photo using the X20 objective & phase microscopy. Right photo X40 obj. & brightfield.**



**AEB 1361. *Saccobolus citrinus*.** Young asci and paraphyses shown mounted in Melzer's reagent using the X40 obj. & brightfield microscopy. Note bluing of the ascus wall.



**AEB 1361. *Saccobolus citrinus*.** Young asci and yellow paraphyses mounted in SMF using the X40 obj. & brightfield microscopy. Note that the unpigmented ascospores are positioned but not cemented together, perhaps sl. squashed apart.



**AEB 1361. Mature *Saccobolus citrinus* asci and cemented clusters of pigmented finely punctate ascospores. A SMF mount seen under the X40 objective using brightfield microscopy. Overall, these and other mature ascospore clusters (minus their gelatinous sheaths) measured  $45\text{--}52.5 \times 15\text{--}19 \mu\text{m}$  (n=15).**



**AEB 1361. Mature *Saccobolus citrinus* asci and cemented clusters of pigmented finely punctate ascospores. SMF mounts seen under the X40 objective using phase (left & right photos) and brightfield (center). Note the short-stalked, apically truncate asci (overall measuring  $(95\text{--})107.5\text{--}117.5\text{--}(132.5) \times (17.5\text{--})22.5\text{--}25 \mu\text{m}$  ( $n=25$ ). An ascus operculum in the left photo is arrowed.**



**AEB 1361. *Saccobolus citrinus* ascospore clusters: Left photo SMF mount using the X40 objective and brightfield microscopy. Right photo the same ascospore cluster, X40 obj. but phase microscopy. Note the cluster gelatinous sheath (clearest under phase), the fine punctations on the apically truncate ascospores and the occasional fissures in their episporium (arrowed). Individual ascospores overall 17.5–20 × 7.5–10 μm (n=25).**