
Studies of coprophilous ascomycetes in Kenya – *Ascobolus* species from wildlife dung

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Species of coprophilous *Ascobolus* were examined in a study of coprophilous fungi in different habitats and wildlife dung types from National Parks in Kenya. Dung samples were collected in the field and returned to the laboratory where they were incubated in moist chamber culture. Coprophilous *Ascobolus* were isolated from giraffe, impala, common zebra, African elephant dung, Cape buffalo, dikdik, hippopotamus, black rhinoceros and waterbuck dung. Six species, *Ascobolus amoenus*, *A. bistisii*, *A. calesco*, *A. immersus*, *A. nairobiensis* and *A. tsavoensis* are identified and described. *Ascobolus calesco*, *A. amoenus* and *A. bistisii* were the most common. Two new species, *Ascobolus nairobiensis* and *A. tsavoensis* are introduced in this paper. In addition, two others, *Ascobolus bistisii* and *A. calesco* are new records in Kenya and are described and illustrated. The diversity of coprophilous *Ascobolus* from wildlife dung in Kenya as deduced from this study is very high.

Key words – *Ascobolus amoenus* – *A. nairobiensis* – *A. tsavoensis* – elephant – moist chambers – national park – zebra

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Introduction

Fungal inventories are important prerequisites for biodiversity conservation. In this study the taxonomy and biodiversity of coprophilous *Ascobolus* Pers. from wild herbivores in Kenya, a neglected area of ecology and conservation, was carried out. Previous studies on coprophilous fungi in Kenya include studies by Minoura (1969), Khan et al. (1972), Carter et al. (1982), Krug et al. (1987), Krug et al. (1989) and Caretta et al. (1998). These studies show that coprophilous ascomycetes species richness and diversity in Kenya is very high and similar to that found in other tropical areas of the world (Richardson 2001).

Dung from wild herbivores was collected

and incubated at room temperature. *Ascobolus* species that sporulated were examined under a stereo microscope. This survey sought to identify and classify *Ascobolus* Pers. found on various dung types in Kenyan wildlife and develop a checklist for *Ascobolus* species associated with different dung types in wildlife.

Ascobolus belongs to the family Ascobolaceae. *Ascobolus* comprises 60 mostly cosmopolitan and coprophilous species that begin to sporulate on dung within the first week of incubation (Bell 2005, Kirk et al. 2008, www.indexfungorum.org, 2012). The pale-yellow luteous apothecioid ascomata are superficial, gregarious, and sessile (Brummelen van 1967, Doveri 2004, Bell 2005), but sometimes

with a short stalk up to 30 mm diameter. Paraphyses are filiform, cylindrical and often embedded in mucus. Asci are usually unitunicate, operculate, saccate-clavate or cylindrical-clavate (Doveri 2004, Bell 2005), with rounded or dome-shaped apex, protruding above surface of hymenium when mature, amyloid in some species and usually 4 or 8-spored. Asci that protrude way beyond the hymenium at maturity, contain thick-walled ascospores when young, a characteristic pore plugging mechanism of septum structure and similar ascus apical chamber and operculum (Brummelen van 1967, Aas 1992, Dissing et al. 1994). Ascospores are single-celled, subglobose to elliptical or oval, thick-walled, sometimes with a gelatinous sheath, ornamented with pigment which is deposited externally; they can be smooth or variously roughened, 2-3-seriate in ascus, ejected singly and are purple or brown (Bell 2005). These species are often saprobic and coprophilous (Bell 2005).

Globally, species of the genus *Ascobolus* have been recorded in Europe (Brummelen van 1967, Mireille et al. 2002, Doveri 2004), the Middle East (Abdullah et al. 1993), Australia and New Zealand (Bell 1983, 2005), Asia (Liou S-C 1977, Wang 2000a), Russia (Prokhorov 1991) and the Americas (Richardson 2008).

In Africa, *A. albidus* P. Crouan & H. Crouan, *A. amoenus* Oudem., *A. degluptus* Brumm., *A. hawaiiensis* Brumm., *A. immersus* Pers. and *A. stictoideus* Speng. have been recorded in Uganda, South Africa and Egypt (Minoura 1969, Ebersohn et al. 1992, 1997, Abdel-Azeem et al. 2005). *Ascobolus americanus* (Cooke & Ellis) Seaver, *A. immersus* Pers., *A. perplexans* Masee & Salmon, *A. viridulus* W. Phillips & Plowr. and *Ascobolus* sp. have been recorded in Kenya in previous studies (Minoura 1969, Caretta et al. 1998).

In this study six *Ascobolus* species are examined and described.

Materials and Methods

Sample collection and incubation

Wildlife dung was collected from National Parks and Reserves in a coastal forest, savannah, wet montane forest and a dry highland forest in Kenya. The collection sites were mapped using a Global Positioning System

device. The criteria for sampling and site selection were habitat type, dung type, topography and vegetation type. Parameters such as sample number, moisture condition of dung, animal species voiding the dung, vegetation type, location, weather details, collector's name and collection date were recorded in a field datasheet. Over-dried dung was moistened with sterile water taking care not to waterlog it because too much water is known to inhibit fungal sporulation (Bell 2005). Fresh dung was taken to the laboratory and placed in moist chambers (moist filter paper in Petri dishes). Each dung sample was examined for signs of sporulation prior to incubation. The incubation was done at room temperature under natural light to induce sporulation of coprophilous *Ascobolus* (Cain 1934, Brummelen van 1967, Lundqvist 1972, Bell 2005).

Coprophilous fungi examination

From the third day of incubation, the moist chambers were examined for fungi sporulation under a stereoscopic microscope. Thereafter, the dung was examined on a regular basis to monitor fungal sporulation and the development of *Ascobolus* fruiting. Thorough examinations commenced immediately after the first *Ascobolus* fruiting body was observed and continued on a regular basis thereby ensuring that fungal sporulation and the development of *Ascobolus* fruiting was well monitored (Doveri 2004, Bell 2005).

The examination of coprophilous *Ascobolus* was carried out by picking individual fruiting bodies with a pair of very fine forceps or a sharp needle and transferring them directly onto a drop of sterile water placed on a glass slide and covered with a cover slip. Gentle pressure was applied on the mount using a rubber of a pencil to extrude the asci, ascospores and paraphyses for microscopic examination (Brummelen van 1967, Bell 2005). Standard stains were employed to help examine important diagnostic fungal structures. Each species was examined in detail and its sample number, dung type, date of incubation, date of examination, tentative name, notes on reaction with stains, habit and color, photograph number and the age of dung recorded in a laboratory data sheet. Small pieces of dung were placed in envelopes and cryotubes for herbarium deposition.

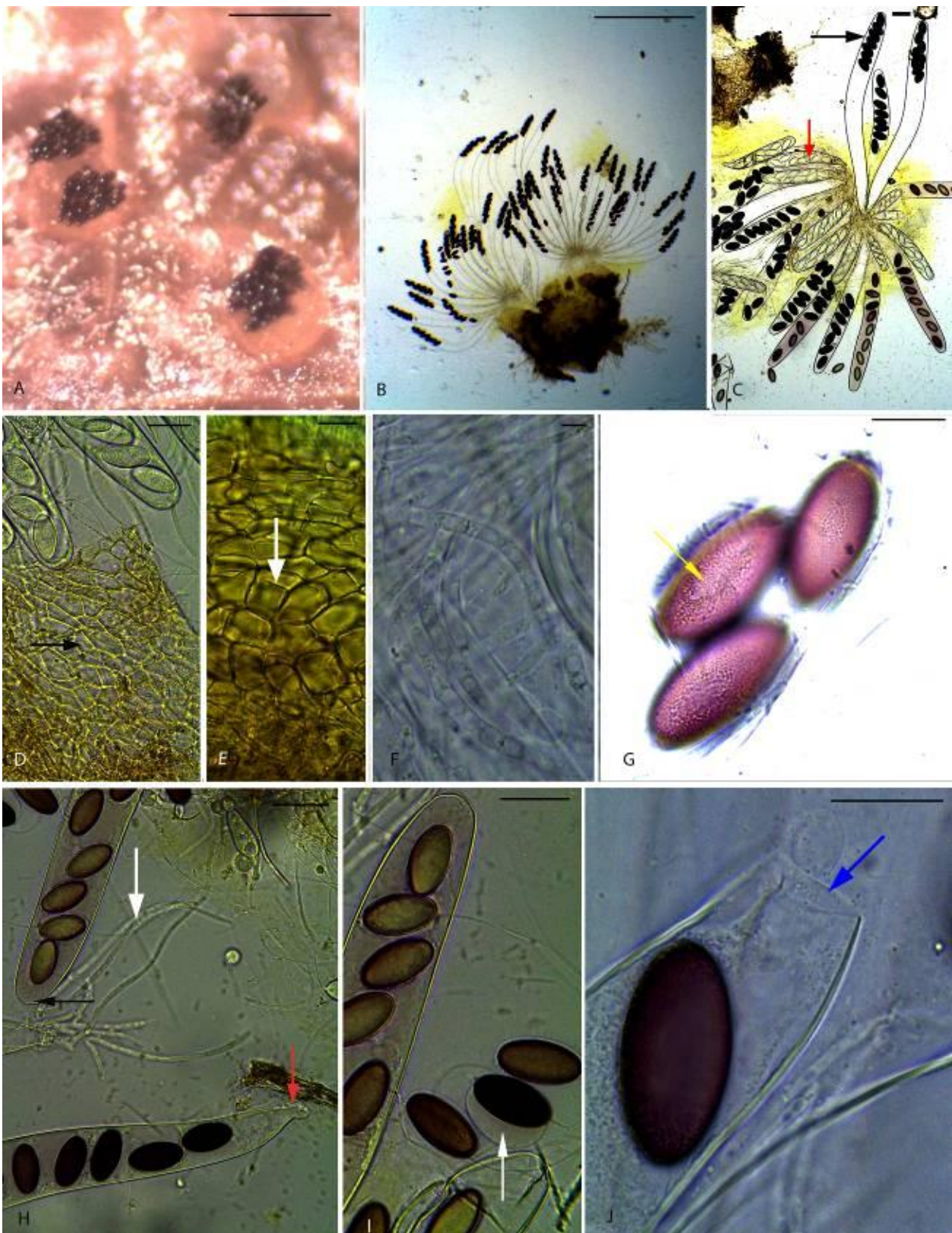


Fig. 1 – *Ascobolus amoenus* (KWSTE003B-2009). **A** Ascomata on substrate. **B** Ascoma squashed in water. **C** Detail of hymenium with 8-spored mature asci (black arrow) and immature asci (red arrow). **D-E** Details of ectal excipulum. Note irregular shape (black arrow) and isodiametric cells (white arrow). **F** Paraphyses. **G** Dotted-granular ascospores. **H** Paraphyses (white arrow) and details of asci, note an ascus apex (black arrow), a short stipitate ascus (red arrow) and the greenish yellow mucus. **I** Mature ascospores, note the unilateral gelatinous sheath (arrow) and greenish mucus. **J** Open operculum (arrow). Scale bars: **A-B** = 500 μ m, **C** = 200, **D, H** = 50 μ m, **E-G, J** = 20 μ m.

Dung fungi specimens were described using features observed under a stereoscopic and compound microscope. Staining of squash mounts was done to enable better observation of micro-structures. Monographs, dichotomous and synoptic keys (Brummelen van 1969, Korf 1972, Richardson et al. 1997, Hansen et al. 1998, Bell 1983, 2005, Doveri 2004) comprising morphological characters such as mycelium, ascomata, vestiture, asci, ascospore, colour, shape, size and formation were extensively used. Comparisons with referenced work were done (Prokhorov 1991, Wang 1993, 1997). Digital photomicrographs were taken on LEICA EZ4D® stereo and LEICA® compound microscope equipped with LEICA Application Suite® (LAS) software. Herbarium specimens and photomicrographs of fungal characters are preserved at the Kenya Wildlife Service.

The dimensions of fungi structures such as apothecia, asci, ascospores and paraphyses were measured using the LAS Suite ® software. A minimum of ten spores and asci were measured and the size range used in descriptions. Detailed examination of micro-morphological characters of asci, ascospores and ascomatal walls was made to enable description and characterization.

Taxonomy

Ascobolus amoenus Oudem., Hedwigia 21: 165 (1882). (Figs. 1A-J)

Ascomata at first cleistothecoid, later apothecioid, solitary or gregarious, superficial or semi-immersed, sessile 350–600 µm high, 200–400 µm diam. *Receptacle* initially closed, later irregularly opening at the top, subglobose to cupulate, smooth, with yellowish granules, emarginate. *Disc* flat to convex, greenish yellow, numerous tips of ripe asci protruding and dotting the hymenium. *Hypothecium* thin composed of slightly oblong cells. *Medullary excipulum* of *textura globulosa*, rather thin cells. *Ectal excipulum* of *textura globulosa-angularis*, yellowish cells 10–33 × 6–10 µm; more regular and isodiametric near the base of receptacle, becoming irregular shaped towards the top, covered by whitish interwoven hyphae. *Paraphyses* filiform, rarely branched at the base, with few septa, hyaline, 2.5–4.0 µm broad, tips rarely inflated, embedded in greenish-yellow mucus. *Asci* 304–416 × 32–39 µm, 8-spored, narrowly clavate,

rounded and curved above, wall bluing in Melzer's reagent, operculum 16–20 µm wide, short-stipitate. *Ascospores* 31–34 × 16–18 µm, initially uniseriate, finally biseriate, ellipsoidal or elongated ellipsoidal, hyaline at first, then violet, finally brownish, smooth to finely punctate or densely granular, with no cracks in the episporium, surrounded by a hyaline gelatinous envelope, thinner on the poles, thicker on the sides.

Material examined – KENYA, Tsavo East National Park Coast Province, six specimens, dung incubated for 11 to 12 days, GPS S3°02 52.3" E038°54 37.0", altitude 354m, African elephant, 27 August 2009, P. Mungai, KWSTE003B-2009; Tsavo East National Park, riverine habitat, GPS S03°02 24.9" E038°42 57.1", altitude 343m, waterbuck, 27 August 2009, P. Mungai, KWSTE006B-2009. Tsavo East National Park, riverine habitat, GPS S03°04 26.9" E038°48 32.3", altitude 305m, Cape buffalo, 27 August 2009, P. Mungai, KWSTE007B-2009; Tsavo East National Park, bushed grassland habitat, GPS S03°02 52.3" E038°54 37.0", altitude 354m, impala, 27 August 2009, P. Mungai, KWSTE002B-2009; Aberdare Country Club Sanctuary, bushed grassland habitat, GPS S00°19 28.1" E036°55 54.3", altitude 2161m, zebra, 30 August 2009, P. Mungai, KWSACC002-2009; Nairobi National Park, savannah grassland, GPS S01°21 21.8" E036°46 51.8", altitude 1765m, 31 August 2009, P. Mungai, KWSNNP007-2009.

Notes – The description of this Kenyan *Ascobolus amoenus* Sect. *Dasyobolus* (Sacc.) Brumm. agrees with those provided for the same species by Brummelen van (1967), Minoura (1969), Jahn (1997) and Bell (2005). Our collection however, differs in the size of important features from the Russian collection of *A. amoenus* (Prokhorov 1991). The Taiwan collection of *A. amoenus* examined by Wang (1997) had broadly clavate asci 395–475 × 33–47 µm, ellipsoidal ascospores, 33–36 × 16–18 µm with unilateral or all-sided gelatinous sheath and paraphyses that were filiform, not enlarged at tips, 2–3 µm wide and embedded in greenish yellow mucus. *A. amoenus* is differentiated from *A. elegans* (J. Klein) Brumm. by its relatively smaller ascospores. This species has previously been recorded in Kenya as *A. americanus* (Minoura 1969).

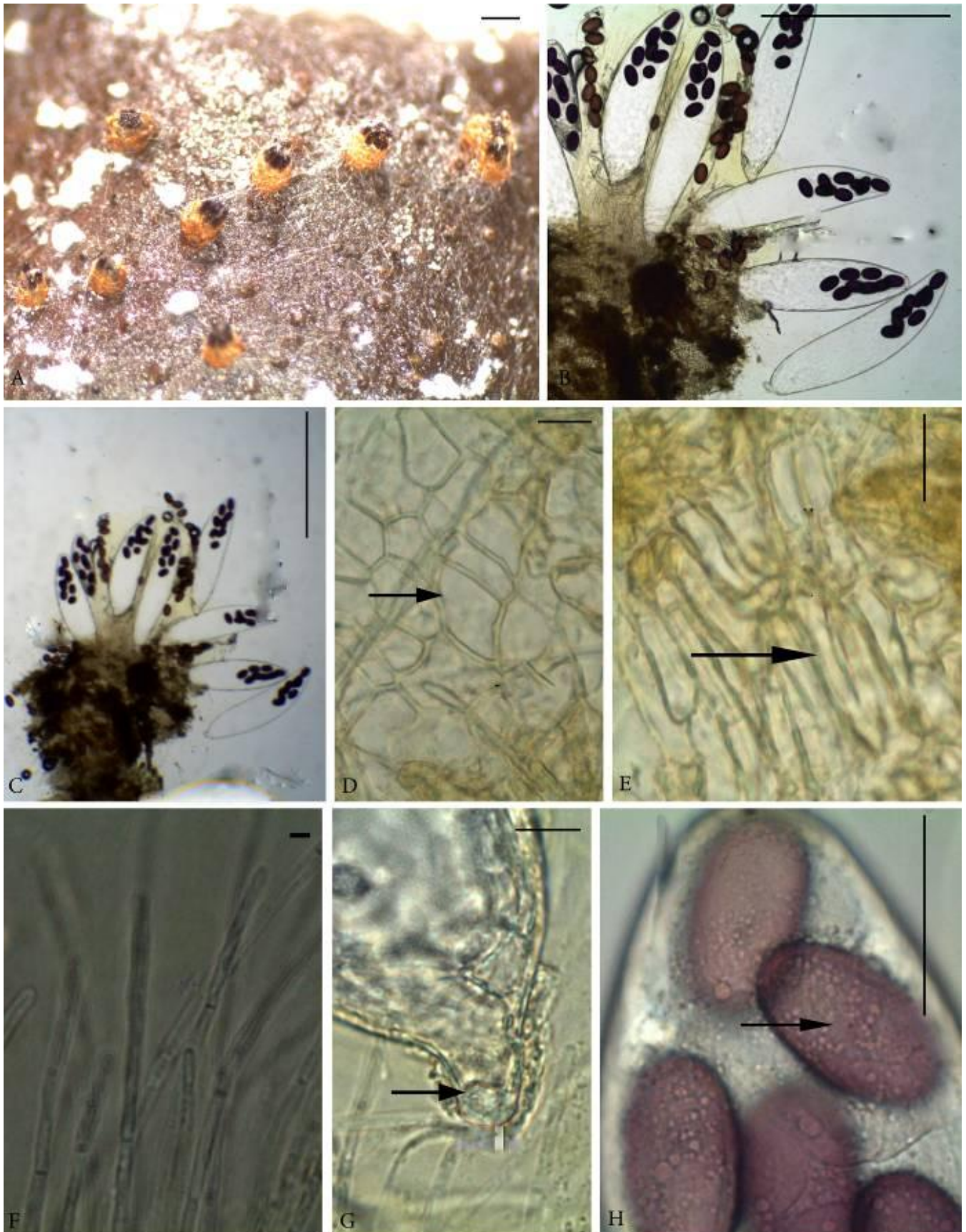


Fig. 2 – *Ascobolus bistisii* (KWSNP020-2010). **A** Ascomata on dung. **B** Mature asci and ascospores. **C** Squashed ascoma. **D-E** Details of ectal excipulum, note shape of cells and elongated cells near the margin (arrows). **F** Paraphyses. **G** Ascus stipe, (arrow). **H** Free mature ascospores, note oil droplets (arrow). **Scale bars:** **A** = 1000 μ m, **B** = 200 μ m, **C** = 500 μ m, **D-H** = 20 μ m

Ascobolus bistisii Gamundí & Ranalli, Nova Hedwigia 10: 347 (1966). (Figs.2A-H & 3I-Q)

Ascomata cleistothecioid in the early stages with a hymenium exposed only late, gregarious, and superficial to semi-immersed, sessile, 200–500 µm diameter. *Receptacle* globular, dotted with few protruding, finger-like asci, light brown at first, finally brown, glabrous, becoming barrel shaped, widest at equatorial part, with a hardly differentiated margin. *Disc* convex, light greenish yellow to brown. *Hypothecium* very thin of isodiametric cells. *Medullary excipulum* of *textura angularis* cells 5–10 × 6–26.5 µm. *Ectal excipulum* of *textura angularis* prismatic cells 26–33 × 4.5–5.5 µm, with hyphoid hairs 34–42 × 4–5 µm. *Paraphyses* cylindric-filiform, more often branched, with numerous septae, exceeding the asci, 2.5–4 µm broad, containing light-refracting granules, tips not inflated, embedded in a light green mucus. *Asci* 312–553 × 87–127 µm, 8-spored, broadly clavate-cylindrical, weakly amyloid, operculate with a dome-shaped apex 30–35 µm wide, abruptly ends into a short stipe, 13–14 µm long. *Ascospores* 50–54 × 30–32 µm, irregularly biseriate, ellipsoidal or sub-cylindrical, rounded at the ends, at first hyaline, then grey, through purple, finally chest nut brown, irregularly distributed episporial pigment forming notable papillate tubercles, with oil droplets, gelatinous sheath notably broader on one side.

Material examined – KENYA, Nairobi National Park, Nairobi Province, four specimens, dung incubated for 10 to 12 days, GPS 37M0255729 9849626, altitude 1680m, bushed grassland, impala, 20 August 2010, P. Mungai, KWSNNP020-2010; Nairobi National Park, Nairobi Province, wooded grassland, GPS 37M0257532 9848948, altitude. 1647m, giraffe, 20 August 2010 P. Mungai, KWSNNP017A-2010; Nairobi National Park, Nairobi Province, wooded grassland, GPS 37M0257082 9850692, altitude 1668m, zebra, 20 August 2010, P. Mungai, KWSNNP018-2010; Nairobi N.P., Nairobi Province, wooded grassland, GPS 37M0257082 9850692, altitude 1668m, giraffe, 20 August 2010, KWSNNP017B-2010.

Notes – *Ascobolus bistisii* Sect. *Dasyobolus* is a fairly common species on Kenyan wildlife dung. This species is morphologically similar to *A. immersus*. However, the smaller sized spores, type of

ornamentation coupled with the unilateral gelatinous sheath points to the species being *A. bistisii* (Richardson 1997, Mireille et al. 2002, Bell 2005, Doveri 2004). This is a new record for Kenya.

Ascobolus calesco A.E. Bell & Mahoney, Fungal Planet, no. 21: 22, (2007). (Figs.4A-K)

Ascomata apothecioid, scattered or gregarious, semi-immersed, sessile, 1100–1360 µm high, 690–910 µm diameter. *Receptacle* whitish to pale ochre yellow, barrel-shaped, with an indistinct margin. *Disc* globular or pyriform, at first closed, later irregularly opening at the top. *Hymenium* plane, dirty white, dotted with few black protruding tips of asci. *Hypothecium* and medullary excipulum not well differentiated. *Ectal excipulum* of *textura angularis* cells 20–37 × 11–19 µm, somewhat horizontally elongated towards the top. *Paraphyses* filiform, hyaline, vacuolated, septate, 3–6 µm width, embedded in yellowish green mucus. *Asci* 180–200 × 50–60 µm, 8-spored, unitunicate, broadly clavate at maturity, wall bluing in Melzer's reagent, operculum 32–50 µm wide, short stipitate. *Ascospores* 43–50 × 31–36 µm, single-celled, ellipsoidal to semi-globose, biseriate, rose colored at maturity, turning brown with age, epispore smooth or fissured, rarely verruculose, roundish at the ends, surrounded by a hyaline gelatinous envelope.

Material examined – KENYA, Tsavo East National Park, Coast Province, seven specimens, dung incubated for 6 to 8 days, GPS S03°00'29.7" E038°04'35.8" altitude 354m, on dikdik, 27 August 2009, P. Mungai, KWSTE005B-2009; Tsavo East National Park, Coast Province, GPS S03°02'52.2" S03°02'52.2", altitude 236m, riverine vegetation, on giraffe, 27 August 2009, P. Mungai, KWSTE008B-2009. Tsavo East National Park, Coast Province, GPS S03°04'26.9" E38°48'32.3", altitude 305m, riverine vegetation, Cape buffalo, 27 August 2009, P. Mungai, KWSTE007B-2009; Aberdare Country Club Game Sanctuary, Central Province, GPS S00°19'28.1" E36°55'54.3", altitude 2161m, bushed grassland, zebra, 30 August 2009, P. Mungai, ACC002. Tsavo East National Park, GPS S03°02'52.3 038°54'37.0, altitude 354m, riverine vegetation, elephant, 27 August 2009, P. Mungai, KWSTE003B-2009. Aberdare National

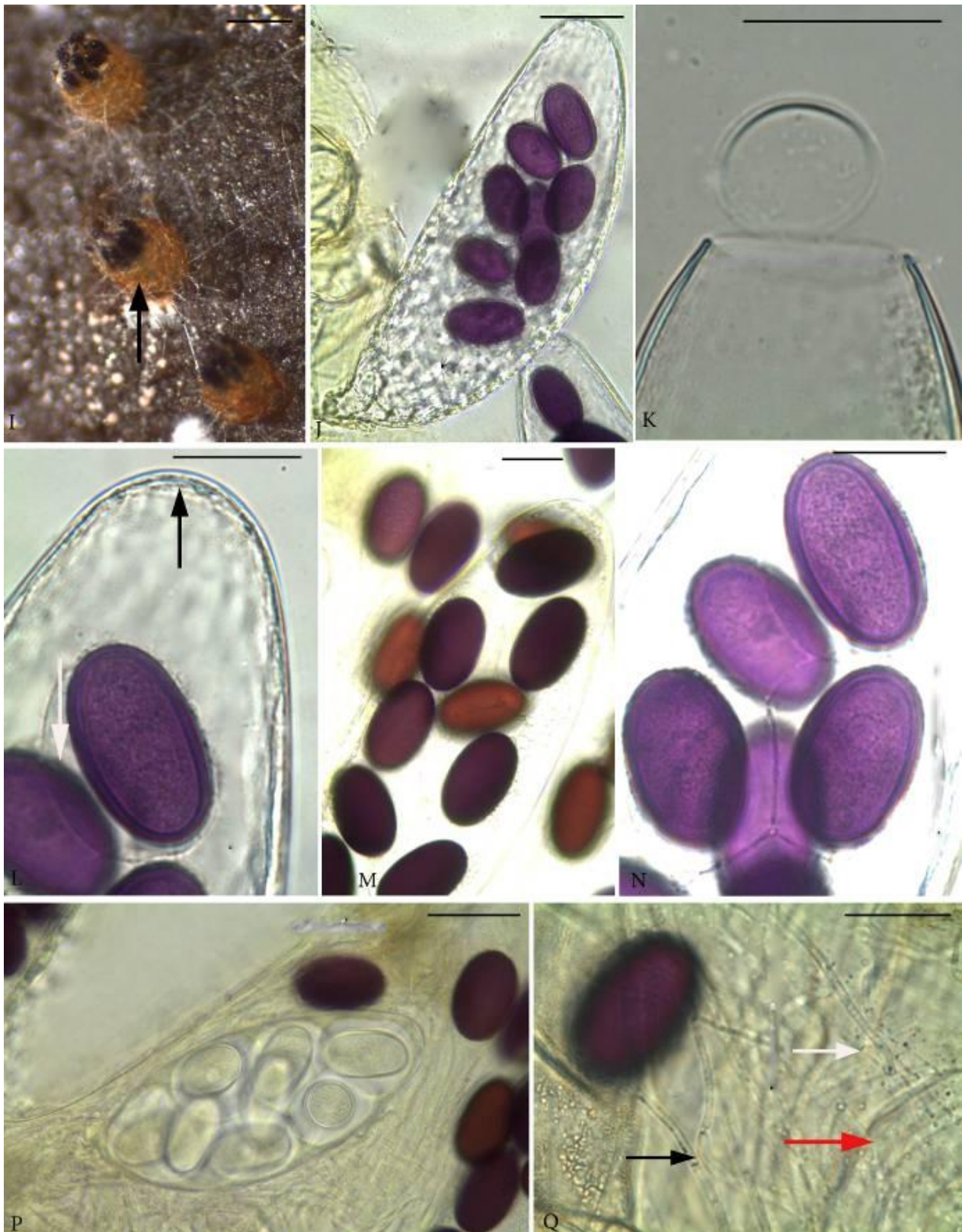


Fig. 3 – *Ascobolus bistisii* (KWSNNP020-2010). **I** Ascomata on dung, note the hyphoid hairs (arrow). **J** Mature ascus with ascospores. **K** Open operculum. **L** Apical portion of ascus, note the closed operculum (black arrow) and unilateral sheath on ascospore (white arrow). **M-N** Ascospores at different stages. **P** Immature ascus with ascospores among mature ascospores. **Q** Paraphyses, note the anastomosing (black arrow), light green mucilage (red arrow) and branching (white arrow). **Scale bars:** **I** = 500 μ m, **J** = 50 μ m, **K-L** = 20 μ m, **M** = 50 μ m, **N** = 20 μ m, **P** = 50 μ m, **Q** = 20 μ m.

Park, Central Province, GPS S00°21'42.0" E036°52'55.9", altitude 2076m, montane forest black rhinoceros, 29 August 2009, P. Mungai, KWSANP004-2009. Nairobi National Park, Nairobi Province, GPS S01°20'50.1 E036°47'51.3, altitude 1695m, savannah grassland, hippopotamus, 31 August 2009, P. Mungai, KWSNNP012-2009.

Notes – *Ascobolus calesco* Sect. *Dasyobolus* is a very common species on various wildlife herbivore dung types in Kenya. In van Brummelen's monograph (1967) and Bell's study (2005), an unvalidated species *Ascobolus quezelii* Faurel & Schotter is mentioned and has descriptions most fitting to this Kenyan collection. Bell et al. (2007) clarified the correct taxonomic position with a new species examined from Australia and named *Ascobolus calesco*. Our Kenyan collection has ascospores within the size range 43–50 × 31–36 µm which compares well with *A. calesco* A.E. Bell & Mahoney. This is a new record for Kenya.

Ascobolus immersus Pers., Neues Mag. Bot. 1:115 (1794). (Figs. 5A-K)

Ascomata cleistothecoid in the early stages, gregarious or scattered, immersed or superficial, sessile, 1600–1700 µm high, 850–900 µm diam. *Receptacle* deep yellow to yellowish-brown, subglobose, globose to turbinate, usually pyriform at maturity, margin not differentiated. *Disc* flat to somewhat undulating, shiny, a few ripe asci protruding above the hymenium. *Hypothecium* very thin, of isodiametric cells. *Medullary excipulum* thin, of *textura globulosa* or *angularis* hyaline cells. *Ectal excipulum* of somewhat horizontally elongated *textura angularis* yellowish-brown thick walled cells, 13–49.5 × 10–22 µm. *Paraphyses* filiform, simple or sparingly branched at the base, septate, exceeding asci, 2–4 µm broad, tips not swollen and rarely uncinuate, embedded in greenish-yellow mucus. *Asci* 445–724 × 97–139 µm, 8-spored, unitunicate, broadly clavate to sacciform, rounded above, wall turning deep blue in Melzer's reagent, operculum ca. 25 µm diam., short stipitate. *Ascospores* 54–64 × 31–35.5 µm, uniseriate to biseriate, single-celled, subcylindrical, ends markedly rounded, at first hyaline, later violet becoming purple-brown at maturity,

at first smooth, later with few anastomosed cracks; gelatinous sheath on each spore, hyaline, broader on sides and narrow on polar region.

Material Examined – KENYA, Nairobi National Park, Nairobi Province, three specimens, dung incubated for 10 to 20 days, GPS 37M0257532 9848948, altitude 1647m, wooded grassland, giraffe, 20 August 2010, P. Mungai, KWSNNP018-2010; Nairobi National Park, Nairobi Province, GPS 37M0255729 9849626, altitude 1680m, impala, 20 August 2010, P. Mungai, KWSNNP020-2010; Nairobi National Park, Nairobi Province, GPS 37M0257532 9848948, altitude 1647m, giraffe, 20 August 2010, P. Mungai, KWSNNP017A-2010.

Notes – *Ascobolus immersus* Sect. *Dasyobolus* is very similar to *A. bistisii* Gamundi & Ranali (Brummelen van 1967, Bell 2005). The two species are however differentiated by having different ascospore shape, ornamentation and size: ascospores of *A. bistisii* are smaller and finely warted (Brummelen van 1967, Mireille et al. 2002, Bell 2005) while those of *A. immersus* are bigger, adorn striations and are more rounded at the ends (Brummelen van 1967, Richardson et al. 1997, Hansen et al. 2001, Doveri 2004, Bell 2005). *A. immersus* is also easily differentiated from other *Ascobolus* species of sect. *Dasyobolus* (cleistothecoid ascomata, opening at late maturity only, or not opening at all) by its comparatively large ascomata, asci and ascospores, the latter typically smooth or with thin crevices (Brummelen van 1967, Doveri 2004). *A. immersus* is a very large and common coprophilous species in Kenya sporulating very early on incubated dung.

New coprophilous *Ascobolus* species from Kenya

Ascobolus nairobiensis Mungai & K.D. Hyde, *sp. nov.* (Figs. 6A-I)

Mycobank: MB564303

Ascomata apothecoid, gregarious, superficial, sessile, 250–400 µm high, 150–250 µm diam. *Receptacle* at first closed, subcylindrical or barrel shaped, cylindrical with an obconical base in later stages, pale brown to brown or white with a pale brownish base somewhat translucent, finally often yellowish

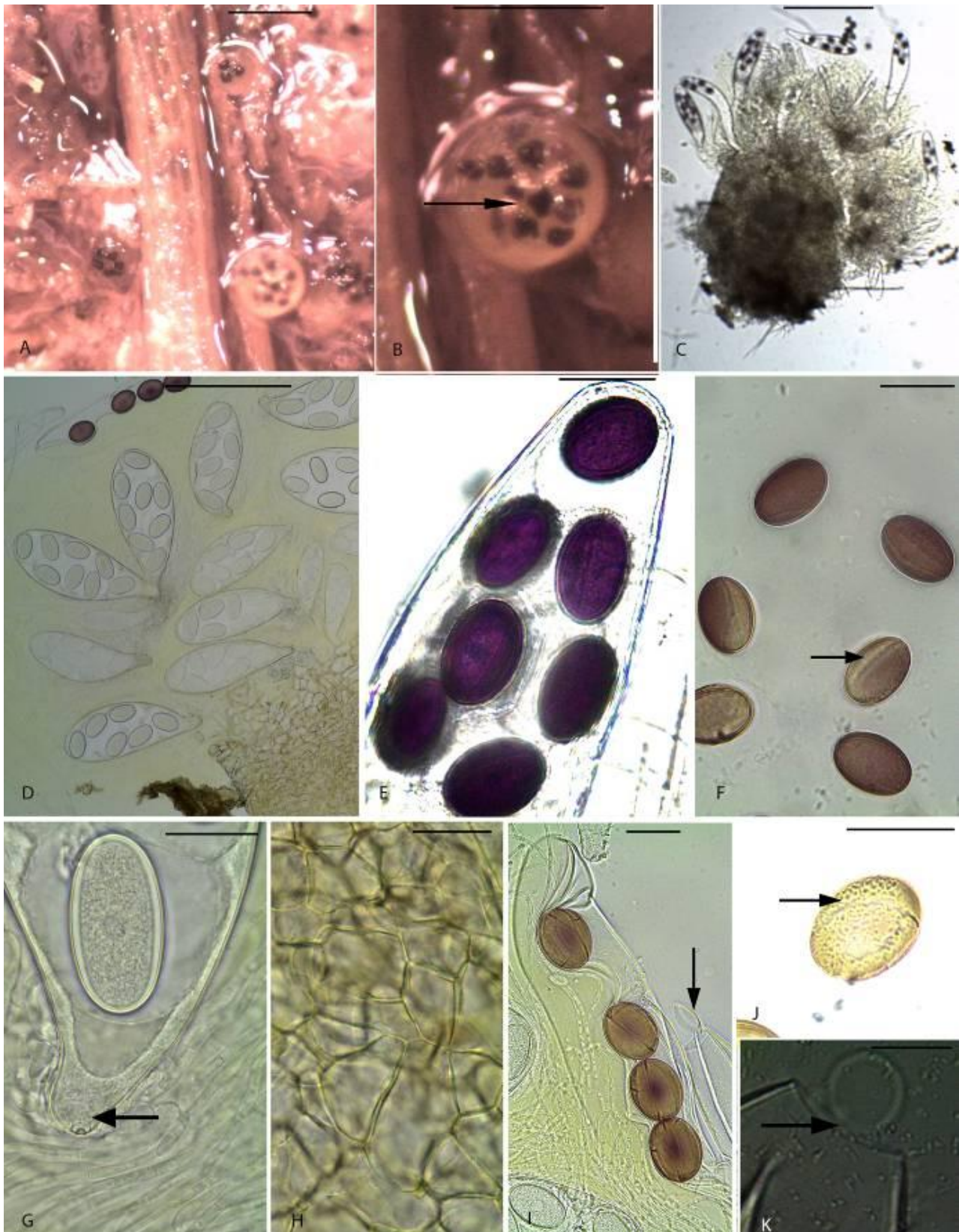


Fig. 4 – *Ascobolus calesco* (KWSTE005B-2009). **A-B** Ascomata on substrate, note black ascus tips (arrow). **C** Ascoma squashed mount. **D** Free asci with spores. **E** Ascus tip with spores. **F** Free ascospores, note ornamentation (arrow). **G** Paraphyses and ascus stipe (arrow). **H** Details of ascomatal wall. **I** Ascus apex showing an open operculum (arrow). **J** Ascospore showing ornamentation (arrow). **K** Ascus apex showing open operculum (arrow). **Scale bars:** **A** = 1000 μm , **B-C** = 500 μm , **D** = 200 μm , **E-F** = 50 μm , **G-H** = 20 μm , **I-K** = 50 μm .

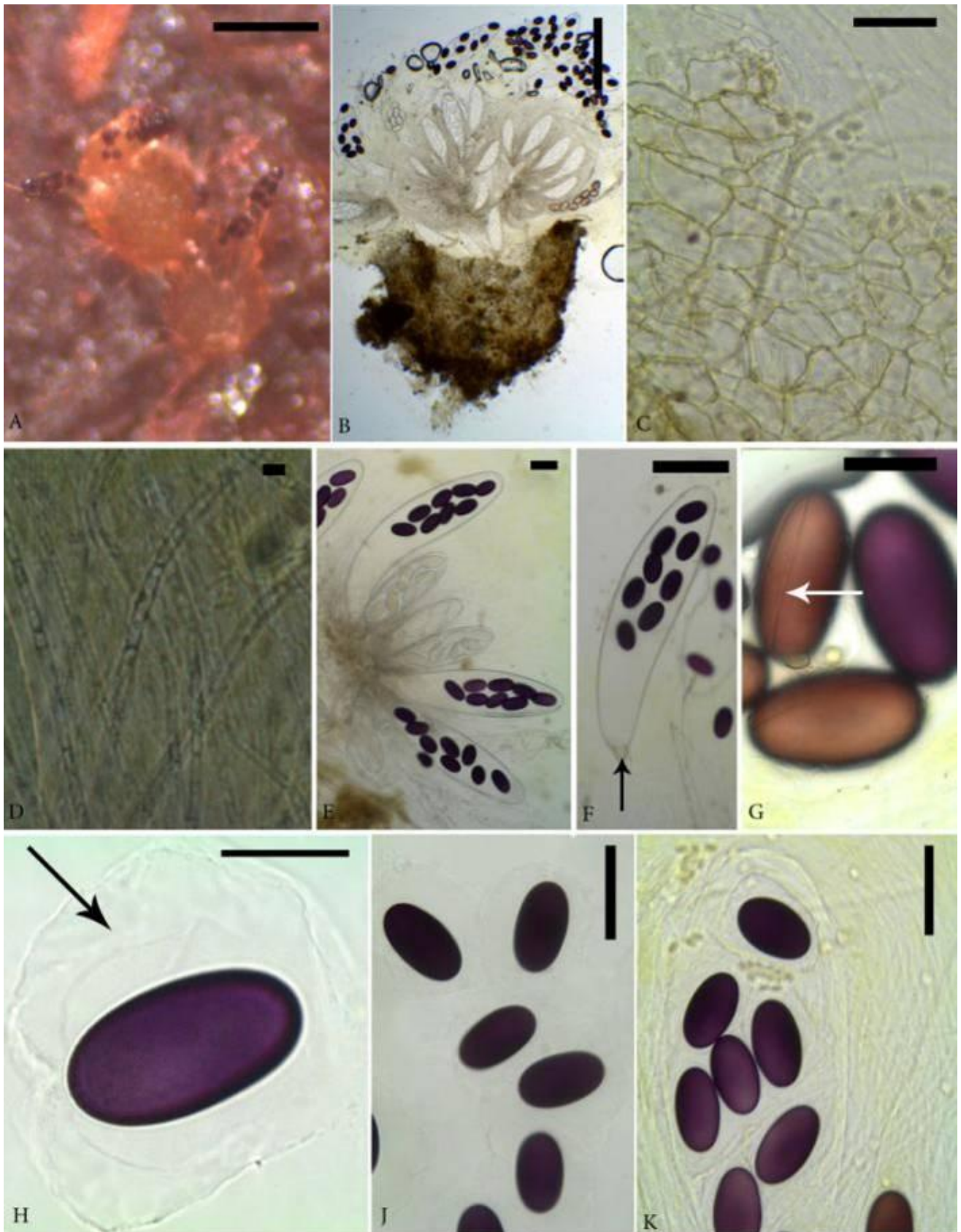


Fig. 5 – *Ascobolus immersus* (KWSNNP020-2010). **A** Ascomata on dung **B** Ascoma squash mount. **C** Details of ectal excipulum. **D** Paraphyses. **E** Immature and mature asci and ascospores. **F** Ascus, note the short stipe (arrow) and apical portion. **G** Ascospores, note striations (arrow). **H** Mature ascospore, note the broad gelatinous sheath (arrow). **J** Free ascospores. **K** Ascospores in the apical part of ascus. **Scale bars:** **A-B** = 500 μ m, **C** = 50 μ m, **D** = 20 μ m, **E-F** = 200 μ m, **G-H** = 20 μ m, **J-K** = 50 μ m.

brown, smooth, emarginate. *Disc* flat, dotted due to the dark pigmented spores inside the protruding asci. *Hypothecium* of *textura angularis* polygonal cells. *Medullary excipulum* of *textura epidermoidea* cells. *Ectal excipulum* of *textura globulosa-angularis* made up of roundish or polygonal pale yellowish cells, $5.5\text{--}16.5 \times 4\text{--}12.5$ μm . *Paraphyses* filiform, simple or sometimes branched, septate, numerous, intertwined, exceeding the asci, $2.5\text{--}4$ μm diam., containing hyaline vacuoles, curved above with slightly swollen tips, embedded in a colorless mucus. *Asci* $160\text{--}190 \times 26\text{--}34$ μm , 8-spored, cylindrical-claviform, rounded above, with the wall staining blue in Melzer's reagent, operculum $9.5\text{--}13.5$ μm diam., with a stipe $40\text{--}60 \times 6.5\text{--}8$ μm . *Ascospores* $21\text{--}26.5 \times 12\text{--}14$ μm , uniseriate to irregularly biseriate, ellipsoidal to narrowly ellipsoid, symmetrical, at first hyaline and guttulate, then pale violet, finally violet to brown. Episporium adorned with numerous thin, longitudinal occasionally anastomosing striae; gelatinous sheath thick, hyaline, unilateral.

Etymology – The species epithet is named after Nairobi National Park, Latinized nairobiensis, the location where it was first examined.

Material examined – KENYA, Nairobi National Park, Nairobi Province, GPS 37M 0255191 9849808, altitude 1693m, bushed grassland, one specimen, black rhinoceros dung, incubated for 10 days, 20 August 2010, P. Mungai, (KWSNNP014-2010 holotype).

Notes – In many respects this species is similar to *A. albidus* and *A. furfuraceous* Pers.: Fr. This species, however, differs from *A. albidus* by its shorter and broader asci and smaller ascospores. According to Brummelen van (1967), *A. albidus* has only biseriate ascospores but our collection has both biseriate and uniseriate ascospores. It differs from *A. furfuraceous* in having smooth, emarginate and usually white rather than furfuraceous and pigmented (yellow to yellowish-green) receptacles with a well differentiated margin and paraphyses embedded in a colourless rather than in a yellowish gelatinous mucus (Doveri 2004). Our taxon *Ascobolus nairobiensis* is also similar to *A. sacchariferus* Brumm., from which it can be distinguished by having constantly whitish saucer-shaped apothecia at maturity and according to Doveri (2004) a preference for growing on cervine dung

and a well delineated granulose margin. This collection does not fit any descriptions from current monographs and keys and is thus described as a new species.

Ascobolus tsavoensis Mungai & K.D. Hyde, *sp. nov.* (Figs. 7A-I)

MycoBank: MB564304

Ascomata apothecioid, scattered or gregarious, superficial, sessile, $350\text{--}400 \times 250\text{--}300$ μm , smooth, whitish. *Receptacle* smooth, whitish, subglobose to pyriform, margin undifferentiated. *Disc* almost flat, narrow, dotted with few black tips of protruding asci. *Hypothecium* thin, composed of small globular cells. *Medullary excipulum* and *ectal excipulum* hardly separated, consisting of *textura globulosa* or *angularis* thin walled polygonal cells $10\text{--}29.5 \times 7\text{--}10$ μm . *Paraphyses* filiform, $2\text{--}3$ μm diam., septate, hyaline, containing pale yellow vacuoles, branched at the base, longer than asci, not inflated at tips, embedded in pale yellow or colorless mucus. *Asci* $200\text{--}250 \times 45\text{--}55$ μm , 8-spored, unitunicate, clavate-sacciform, turning deep blue in Melzer's reagent, operculate, with a dome-shaped apex, broad short stipe. *Ascospores* $26\text{--}34 \times 15\text{--}18$ μm , biseriate or irregularly placed, single-celled, ellipsoidal, with rounded ends, at first hyaline, maroon or dark violet at maturity, thick walled, coarsely verrucose, with a unilateral gelatinous sheath.

Etymology – The species epithet is named after Tsavo East National Park, Latinized to tsavoensis, the location where it was first examined.

Material examined – KENYA, Tsavo East National Park, Coast Province, GPS S03°002'24.9" E08°042'57.1", altitude 343m, one specimen, on waterbuck dung, incubated for 11 days, 27 August 2009, P. Mungai, (KWSTE006B-2009 holotype).

Notes – *Ascobolus tsavoensis* is very similar to *A. stictoideus* and *A. degluptus* but it has larger asci and ascospores while the episporial pigment is unevenly distributed (Brummelen van 1967). This taxon has smaller ascomata and ascospores than *A. bistisii*. Owing to these differences, *A. tsavoensis*, though having many features in common with *A. stictoideus* (Bell 2005) is certainly not the same taxon. This collection constitutes a new species.

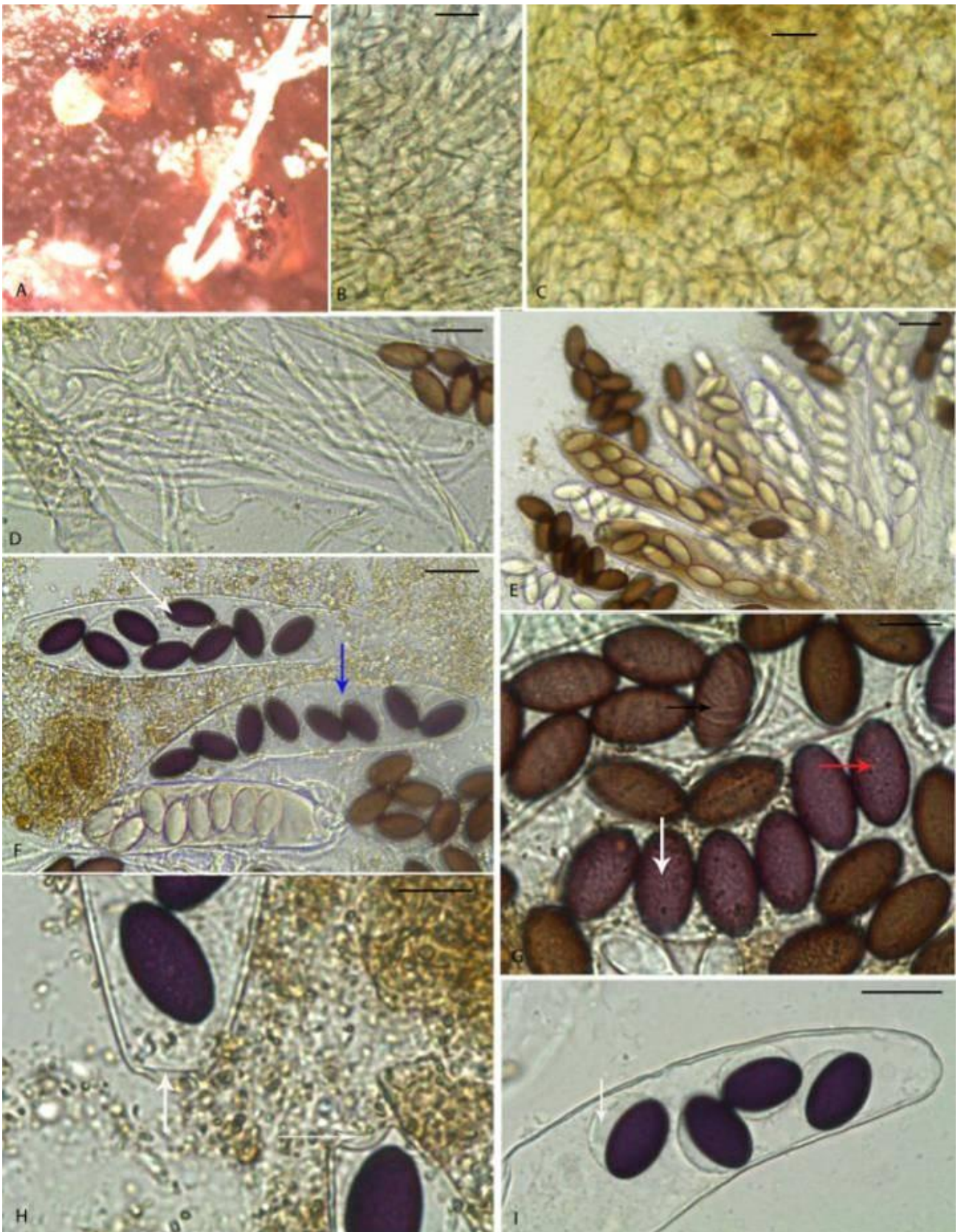


Fig. 6 – *Ascobolus nairobiensis* (KWSNNP014-2010). **A** Ascomata on dung. **B-C** Details of ectal excipulum. **D** Paraphyses. **E-F** Asci with ascospores in different stages, note spore arrangement (white and blue arrow). **G** Free mature ascospores, note the ornamentation (arrows). **H** Apical part of asci, note the opercula (arrows). **I** Ascus apex with ascospores, note unilateral gelatinous sheath (arrow). **Scale bars:** **A** = 1000 μm , **B-C** = 20 μm , **D-F** = 50 μm , **G-I** = 20 μm .

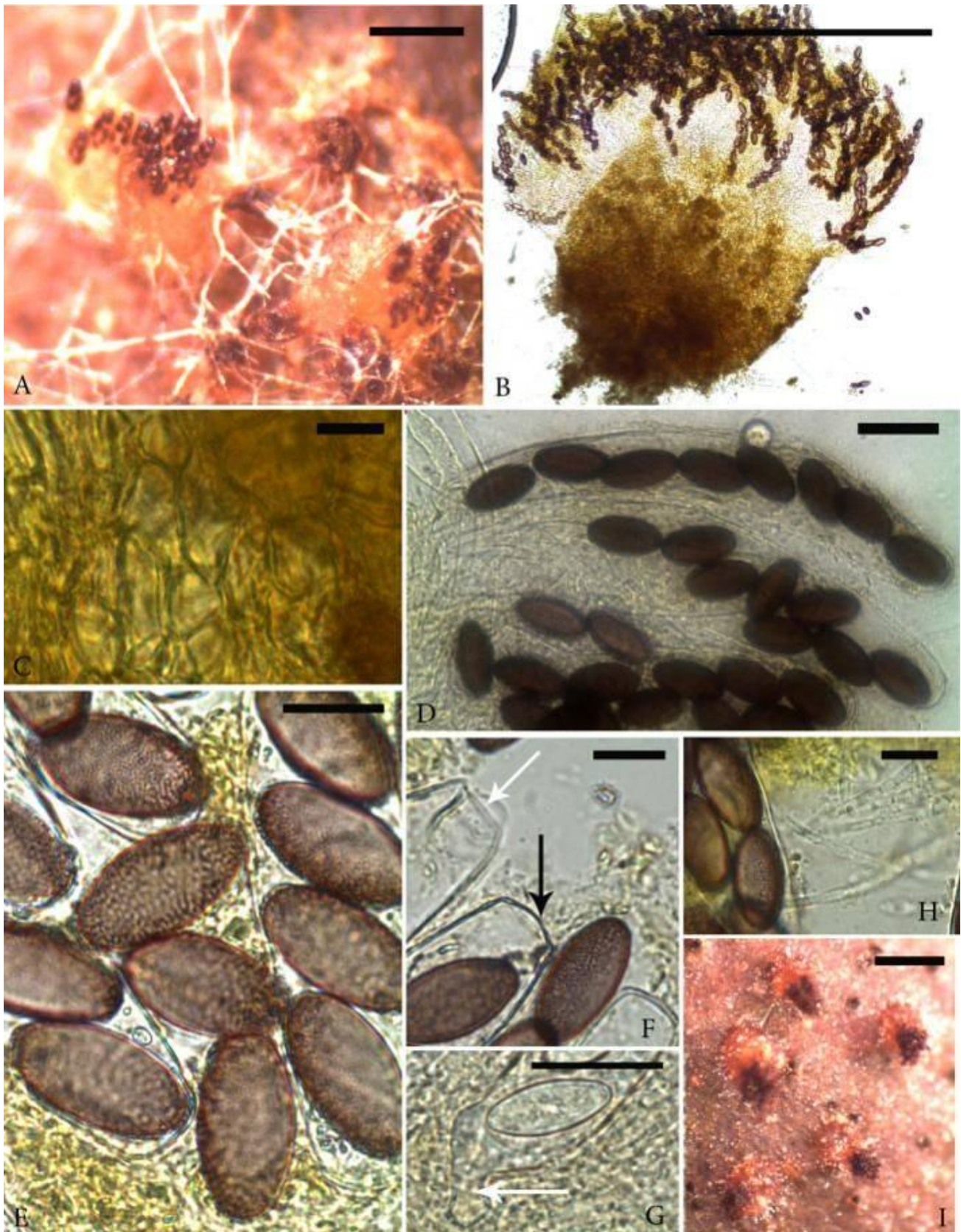


Fig. 7 – *Ascobolus tsavoensis* (KWSTE006B-2009). **A, I** Ascomata on dung. **B** Squashed ascoma. **C** Details of ectal excipulum. **D** Mature Asci with ascospores. **E** Mature ascospores showing thick warts. **F** Asci tips showing open (white arrow) and closed operculum (black arrow). **G** Immature ascus, note stipe (white arrow). **H** Paraphyses and ascospores. **Scale bars:** **A** = 500 μm , **B** = 200 μm , **C** = 20 μm , **D** = 50 μm , **E** = 20 μm , **F** = 20 μm , **G** = 20 μm , **H** = 20 μm , **I** = 1000.

Discussion

Ascobolus and *Saccobolus* belong to the family Ascobolaceae. These two species are morphologically close. They are differentiated by the clustered ascospores in the latter. Most of our species had the typical pale-yellow, luteous, superficial, gregarious and sessile ascomata, similar to observations made by Brummelen van (1967), Doveri (2004) and Bell (2005). Paraphyses were mainly filiform, cylindrical and often embedded in pigmented mucus. Most of our specimens had asci that were unitunicate, operculate, saccate-clavate or cylindric-clavate with rounded or dome-shaped apex, protruding above surface of hymenium when mature, amyloid in some species and 8-spored.

Our specimens had single-celled ascospores that were subglobose to elliptical or oval, thick-walled, sometimes with a gelatinous sheath, ornamented with an externally deposited pigment, smooth or in various degrees of roughness, 2-3-seriate in ascus, blastically ejected singly and are purple or brown (Bell 2005).

Two species, *Ascobolus nairobiensis* and *Ascobolus tsavoensis* do not fit into any existing descriptions in literature and are therefore proposed as new species. In addition, two species, *A. bistisii* and *A. calesco*, have not been recorded in Kenya before.

Ecology

Twenty two isolates of coprophilous *Ascobolus* species sporulated on Kenyan wildlife dung. Zebra had 5 isolates; giraffe had 4, impala 3, waterbuck 2, elephant 2, Cape buffalo 2, dikdik 1, hippopotamus 1 and black rhinoceros 2. *Ascobolus calesco*, *A. amoenus* and *A. bistisii* sporulated on a wide range of dung types. *Ascobolus calesco*, *A. amoenus* and *A. bistisii* were the most common.

Ascobolus bistisii and *A. immersus* sporulated only in fresh dung substrates of wildlife from Nairobi National Park. Old dung from other parks did not yield these species perhaps because it had to be preserved and transported over long distances before incubation and thereby degrading fungi ascospores. On the other hand, dung substrates from Nairobi National Park, being situated close to the laboratory, were always incubated on the same day they were collected from the field. Probably

preservation has an influence on ascospore viability for these taxa.

It was surprising to find giraffe dung among samples yielding a high diversity of fungi. Giraffe, a browser in the African wooded grasslands, is known to feed on bushes, shrubs and short trees usually above three meters high. Interestingly there are no documented coprophilous fungi able to shoot their ascospores to the height of a giraffe's feeding height of browse plants. We hypothesize that perhaps the giraffe gets fungi spores with its intake of water and minerals from natural salt licks.

Kenya is endowed with numerous species of wild animals that comprise diverse species of arthropods, reptiles, birds and mammals. These offer a rich habitat and an untapped reservoir for the study of coprophilous *Ascobolus*. Wildlife dung as a substrate is relatively a virgin area as far as coprophilous fungi are concerned. It, no doubt, provides a relatively unexplored substrate with great potential for many novel *Ascobolus* species to be characterized. Going by the results of this study, the diversity of coprophilous *Ascobolus* from wildlife has been shown to be relatively higher than that from livestock.

Our study was not exhaustive due to limitations of time and key resources. It is recommended that a follow-up detailed taxonomy supported by a phylogenetic study of wildlife dung *Ascobolus* be conducted.

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