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## Coprophilous ascomycetes in Kenya: *Saccobolus* species from wildlife dung

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The taxonomy, occurrence and distribution of *Saccobolus* species was investigated from wild herbivore dung types in Kenya. Dung samples incubated in a moist chamber culture were examined for fungi over three months. Seven species, *Saccobolus citrinus*, *S. depauperatus*, *S. diffusus*, *S. infestans*, *S. platensis*, *S. truncatus* and *S. versicolor* were isolated from African elephant, black rhinoceros, Cape buffalo, dikdik, giraffe, hartebeest, hippopotamus, impala, waterbuck and zebra dung. Five taxa, *S. citrinus*, *S. diffusus*, *S. infestans*, *S. platensis* and *S. truncatus*, are new records for Kenya. The most common taxa were *S. depauperatus* and *S. citrinus*. The diversity of coprophilous *Saccobolus* species in wildlife dung is very high.

**Key words** – African elephant – diversity – moist chambers – national parks – *Saccobolus citrinus*

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### Introduction

The genus *Saccobolus* Boud. (subfamily Ascobolideae Gray, family Ascobolaceae Sacc.) contains over 25 species (Kirk et al. 2008). The main features of this genus include apothecioid ascomata, clustered ascospores, which in most cases have brown or purple-brownish episporic pigment at maturity (van Brummelen 1967, Kaushal & Virdi 1986, Doveri 2004, Bell 2005), with each cluster enclosed in a common gelatinous membrane. The firmly clustered bundles of ascospores are usually arranged in regular patterns (van Brummelen 1967). *Saccobolus* is related to *Ascobolus* Pers. from which it is differentiated by its clustered ascospores. Another important difference between *Saccobolus* and *Ascobolus* is the shorter but broader asci and ascospores of *Saccobolus* (van Brummelen 1967, Doveri 2004, Bell 2005). The apices of paraphyses usually produce a coloured pigment (van Brummelen 1967, Doveri 2004, Bell 2005).

The asci are phototropic, operculate and protrude above the hymenial surface on maturity. van Brummelen (1967) divided *Saccobolus* into two distinct sections, *Saccobolus* whose receptacles and discs are always yellow or amber with yellow or pigmented paraphyses and *Eriobolus* with white, pale violet, violet or brown receptacles and discs and usually colourless paraphyses. *Saccobolus* comprises predominantly cosmopolitan and coprophilous species (van Brummelen 1967, Doveri 2004). Numerous *Saccobolus* species have been recorded globally, for instance, Europe (van Brummelen 1967), Australia and New Zealand (Bell 1983, 2005), Taiwan (Wang 2000) and South America (Richardson 2008). In Africa, records of *Saccobolus* include, *S. beckii* Heimerl., *S. glaber* (Pers.) Lambotte, *S. minimus* Velen., *S. portoricensis* Seaver and *S. verrucisporus* Brumm from South Africa (Ebersohn & Eicker 1992) while *S. citrinus* Boud &

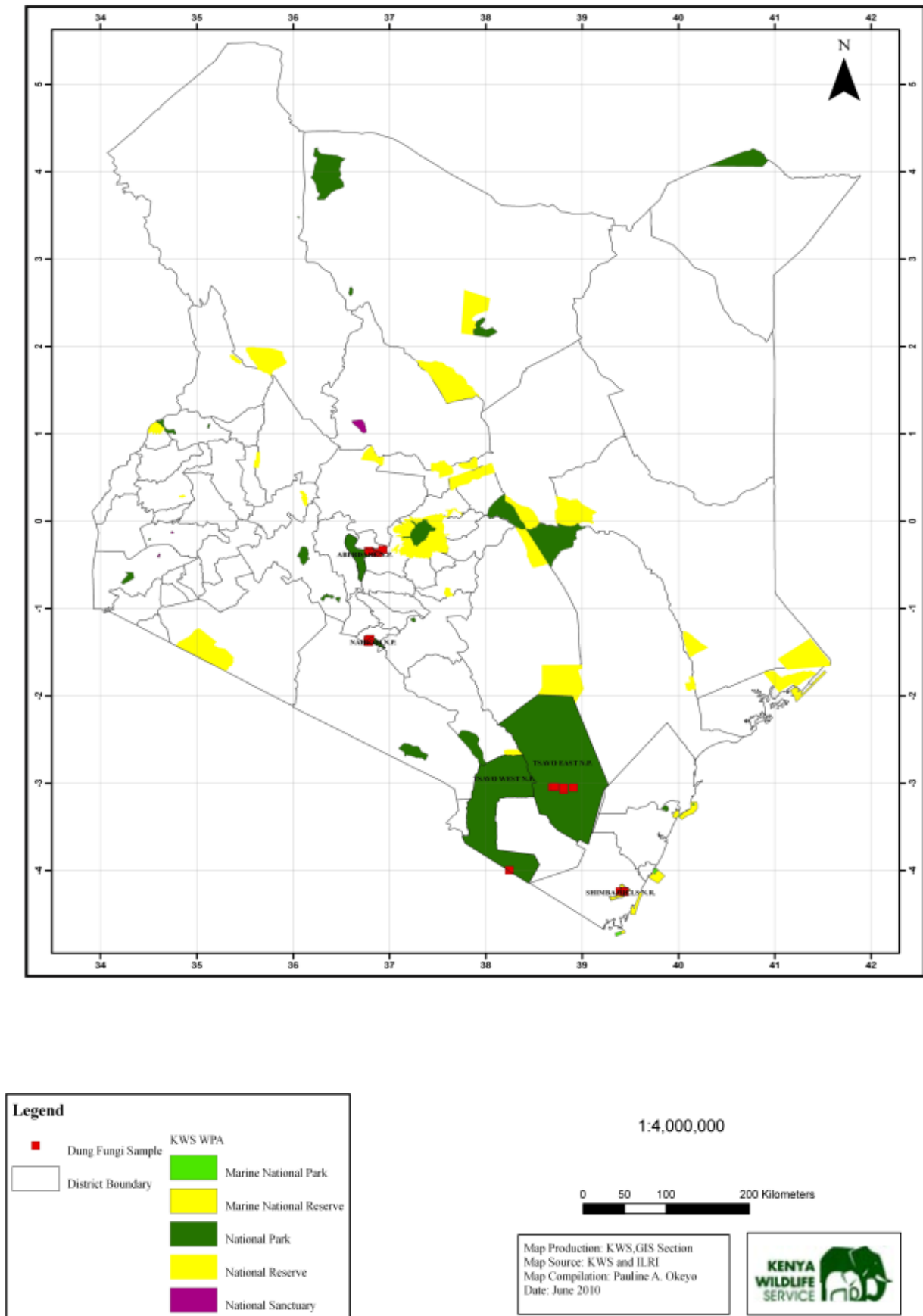


Fig 1. Map of Kenya showing wildlife dung sampling sites

Torrend and *S. glaber* were recorded from Egypt (Abdel-Azeem et al. 2005). From Kenya Minoura (1969) and Caretta et al. (1998) recorded *S. depauperatus* (Berk. & Broome) Rehm, *S. versicolor* (P. Karst.) P. Karst. and *S. violascens* Boud.

The objectives of the present survey were to 1) describe and classify *Saccobolus* species found on various dung types from Kenyan wildlife and, 2) document species diversity and distribution of *Saccobolus* species associated with different dung types.

In this study seven species of *Saccobolus* from wildlife dung are examined and illustrated.

### Materials and Methods

For a detailed methodology refer to our earlier work on coprophilous *Ascobolus* in Kenya (Mungai et al. 2012). The sampling sites are shown in Fig. 1.

### Taxonomy of *Saccobolus* species from Kenyan wildlife dung

*Saccobolus citrinus* Boud. & Torrend, Bull. Soc. Mycol. Fr. 27: 131, 1911. (Figs. 2A–I & 3A–F)

*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* cylindrical-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,

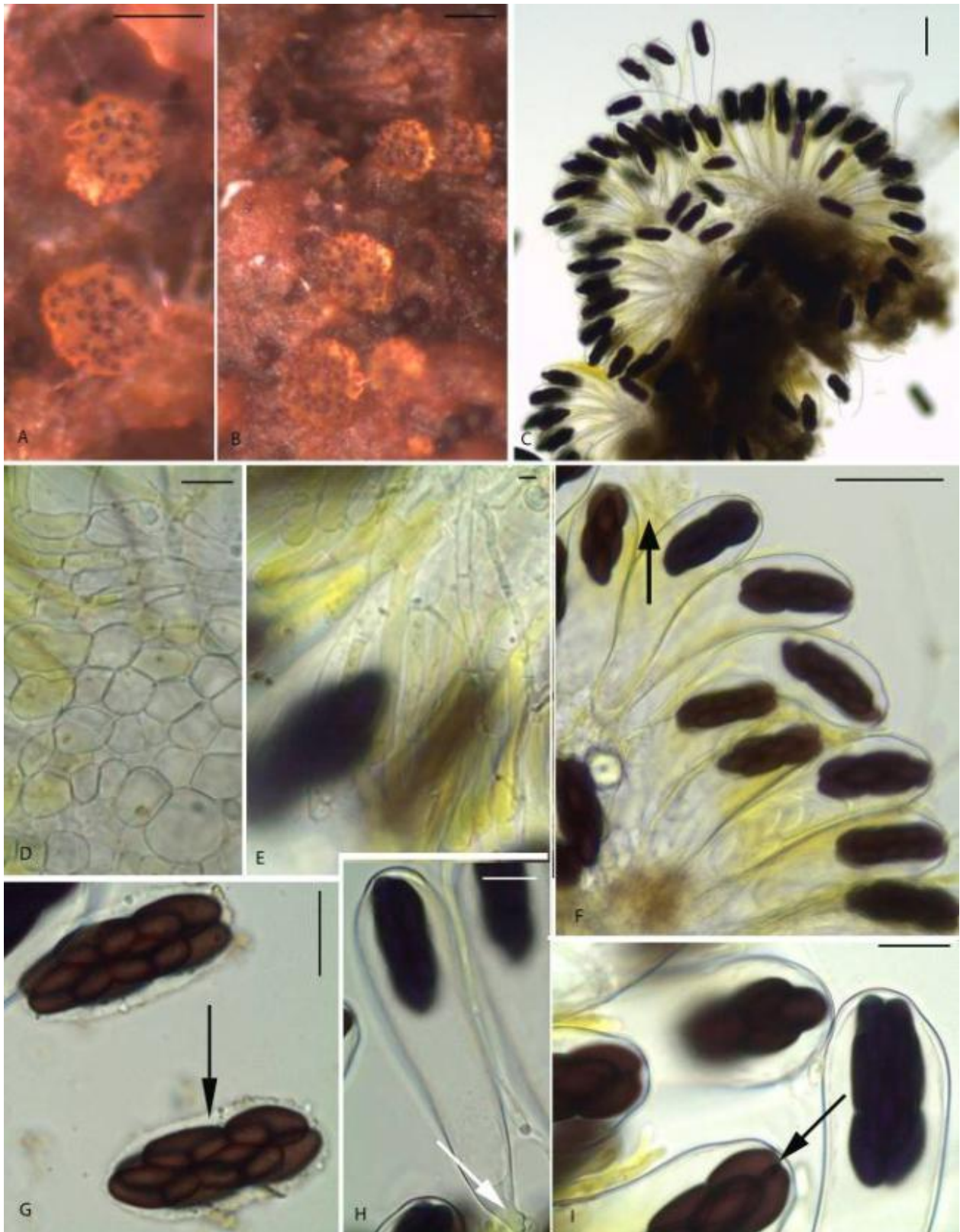
thick-walled; clusters elongated, 45–50.5 × 15–20 µm, compact and firmly enclosed all round in a narrow gelatinous envelope 2–4 µm thick.

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.

*Saccobolus depauperatus* Berk. & Broome., Ann. Mag. Nat. Hist. III 15: 448, 1865. (Figs. 4A–G, 5A–C)

*Ascomata* apothecioid, gregarious, superficial, sessile, 180–300 µm diam. *Receptacle* globose in the early stages, pulvinate to subglobose later, dirty white to pale violet, with a narrow base, smooth, margin not distinct. *Disc* convex, white, dotted with pale violet tips of protruding ripe asci, outer surface somewhat glabrous. *Hymenium* thick. *Hypothecium* thin, of small very compacted isodiametric cells, not well delineated. *Medullary excipulum* thick, made up of small *textura globulosa* cells. *Ectal excipulum* of thin, *textura globulosa-angularis* cells 7–11 × 5.5–10 µm, intercellular pigment not conspicuous amongst these cells. *Paraphyses*



**Fig. 2** – *Saccobolus citrinus* (KWSNNP020-2010). **A-B** Ascomata 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, J = 50  $\mu$ 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.

cylindric-filiform, septate, exceeding the asci, width 2.5–4  $\mu\text{m}$ , with hyaline vacuoles; tips hyaline, not inflated. *Asci* 62–72  $\times$  15.5–18.5  $\mu\text{m}$ , 8-spored, unitunicate, claviform, often curved at apex, wall turning deep blue in Melzer's reagent, with an operculum ca. 14.5  $\mu\text{m}$ , long stipitate. *Ascospores* 11.5–14  $\times$  5–7  $\mu\text{m}$ , single-celled, arranged according to van Brummelen pattern II, ellipsoidal-subfusiform, somewhat asymmetrical, round or truncate at the ends, purple-brown at maturity, smooth or finely dotted; clusters compact, 31.5–35  $\times$  11.5–13.5  $\mu\text{m}$ , usually surrounded by a thin hyaline gelatinous sheath.

Material examined (10 isolates) – KENYA, Shimba Hills National Reserve, Coast Province, GPS S04°13'35.6" E039°25'31.6", altitude 373m, giraffe dung, 26 August 2009, Paul Mungai, KWSSH001-2009; Tsavo East National Park, Coast Province, GPS S03°21'06.4" E038°37'50.1", altitude 514m, riverine vegetation, Cape buffalo dung, 23 August 2008, Paul Mungai, KWSTE008A-2008; Aberdare Country Club Game Sanctuary, Central Province, GPS S00°19'28.1" E036°55'54.3", altitude 2161m, bushed grassland, zebra dung, 30 August 2009, Paul Mungai, KWSACC002-2009. Nairobi National Park, Nairobi Province, GPS S01°21'15.1", E036°46'54.1", altitude 1768m, savannah grassland, impala dung, 31 August 2009, Paul Mungai, KWSNNP008-2009; Tsavo East National Park, Coast Province, GPS S03°02'29.7" E038°41'35.8", altitude 354m, riverine vegetation, dikdik dung, 27 August 2009, Paul Mungai; KWSTE005B-2009; Aberdare Country Club Game Sanctuary, Central Province, GPS S00°19'28.1 E036°55'54.3", altitude 2161m, bushed grassland, giraffe dung, 30 August 2009, Paul Mungai, KWSACC003-2009; Aberdare National Park, Central Province, GPS S00°21'35.0" E036°52'47.8", altitude 2074m, montane forest, African elephant dung, 30 August 2009, Paul Mungai KWSANP003-2009; Tsavo East National Park, Coast Province, GPS S03°21'66.6" E038°38'77.2", altitude 514m, riverine vegetation, dikdik dung, 23 September 2008, P. Mungai, KWSTE005A-2008; Shimba Hills National Reserve, Coast Province, GPS S04°14'35.4" E039°26'07.2", altitude 361m, hartebeest dung, 26 August

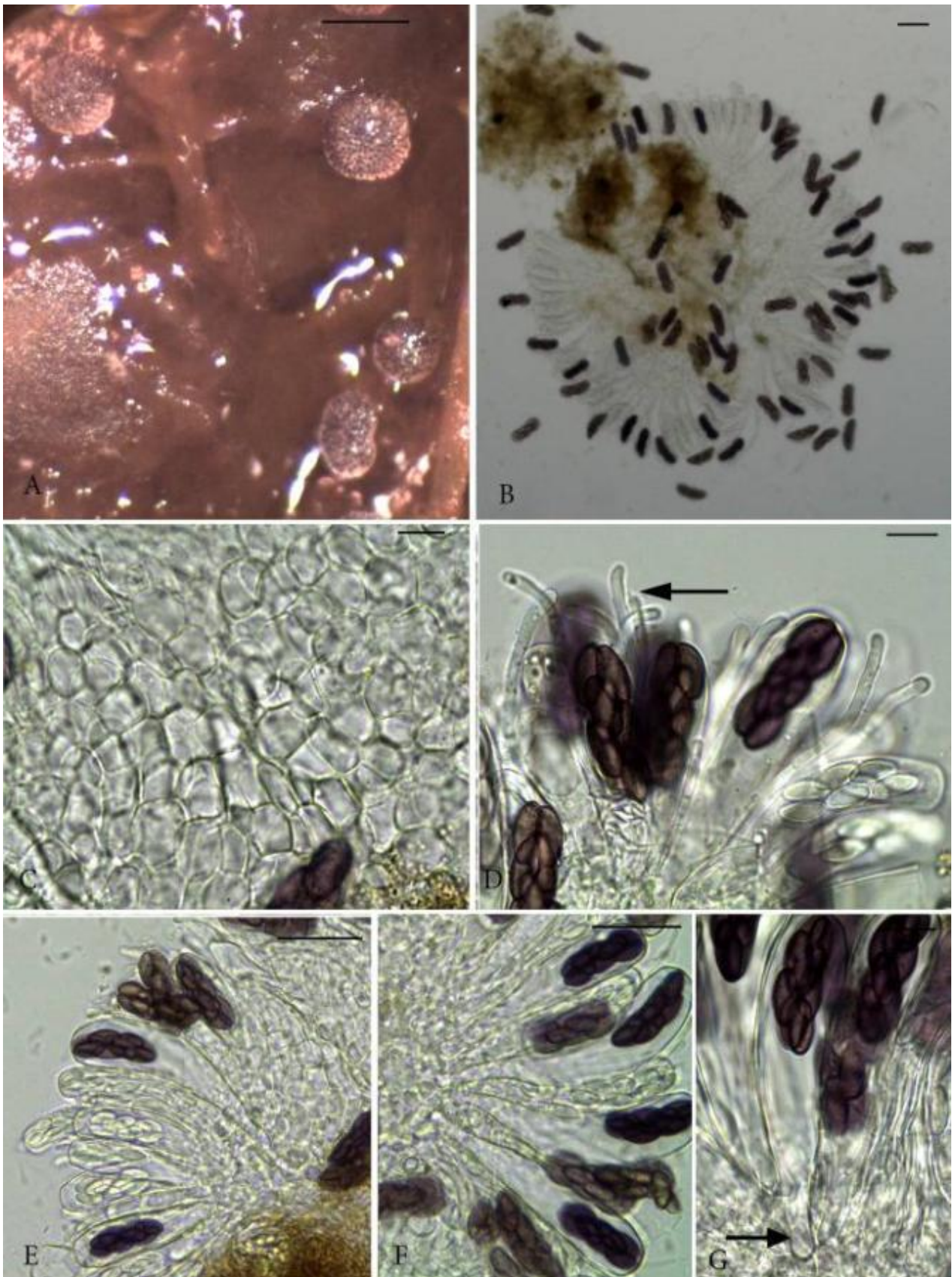
2009, Paul Mungai, KWSSH003-2009; Kinondo Forest Reserve, Coast Province, GPS S04°25'19.7" E039°32'60.2", altitude 6m, coastal tropical rain forest, dikdik dung, 19 April 2009, P. Mungai, KWSKIN004-2009; Nairobi National Park, Nairobi Province, GPS S01°21'25.5", E036°47'51.6", altitude 1748m, savannah grassland, giraffe dung, 31 August 2009, Paul Mungai, KWSNNP009-2009.

Notes – *Saccobolus depauperatus* Sect *Eriobolus* Sacc. is similar to *S. versicolor* (van Brummelen 1967, De Sloover 2002, Doveri 2004). The two species however have some important differences that help in their differentiation. The latter has larger apothecia, asci, single ascospores and ascospore clusters (van Brummelen 1967, Doveri 2004). The excipulum of *S. depauperatus* is usually of a *textura globulosa* structure unlike that of *S. versicolor* which is *textura intricata* (van Brummelen 1967, Doveri 2004). The apices of paraphyses and excipular cells of *S. depauperatus* are also without any pigmented intercellular mucus rendering the species to appear whitish (van Brummelen 1967, De Sloover 2002, Doveri 2004).

***Saccobolus diffusus*** S.C. Kaushal & Viridi, Willdenowia 16(1): 269, 1986. (Figs. 6A–J, 7K–R)

*Ascomata* apothecioid, scattered or gregarious, superficial, sessile, 220–370  $\mu\text{m}$  diam. *Receptacle* at first subglobose to globose, obconic, finally pulvinate, greenish yellow to pale, external surface glabrous, margin entire. *Hymenium* convex, dotted with black tips of mature protruding ripe asci. *Hypothecium* thin, of small *textura globulosa* cells. *Medullary excipulum* not well delineated. *Ectal excipulum* of *textura angularis-globulosa* cells 8–10  $\times$  4.5–8  $\mu\text{m}$ . *Paraphyses* cylindric-filiform, simple or rarely branched, septate, exceeding asci, 2.5–4  $\mu\text{m}$  broad; tips with abundant greenish yellow pigmentation, not inflated. *Asci* 114–167  $\times$  29–39  $\mu\text{m}$ , 8-spored, walls turning blue in Melzer's reagent, unitunicate, broadly clavate, tapering abruptly into a short stout and lobate stipe 13–25  $\times$  6.5–7.5  $\mu\text{m}$ ; apices subtruncate with an operculum 14.5–17  $\mu\text{m}$  wide. *Ascospores* 21–24.5  $\times$  8–9.5  $\mu\text{m}$ , single-celled, ellipsoidal, at first hyaline, violet to brown, smooth or verruculose,





**Fig. 4** – *Saccobolus depauperatus* (KWSSH003-2009). **A** Ascomata on dung. **B** Squashed ascoma. **C** Details of ectal excipulum near the margin. **D** Paraphyses, asci and ascospores (arrow). **E-F** Details of cells of the ectal excipulum seen from outside, medullary excipulum, mature and immature asci and ascospores. **G** Asci stipe (arrow). Scale bars: A = 500  $\mu\text{m}$ , B = 200  $\mu\text{m}$ , C = 20  $\mu\text{m}$ , D = 20  $\mu\text{m}$ , E-F = 50  $\mu\text{m}$ , G = 20  $\mu\text{m}$ .





**Fig. 5** – *Saccobolus depauperatus* (KWSSH003-2009). **A** Asci and ascospores. **B** Details of mature and immature ascospores. **C** Mature and immature asci and ascospores. Scale bars: A = 50  $\mu$ m, B = 20  $\mu$ m, C = 50  $\mu$ m.



arranged according to van Brummelen pattern I, clusters compact, 50–58 × 16–18 µm, more pointed on one side, rather narrow gelatinous sheath of 2–4 µm on polar region and 4–8 µm on sides.

Material examined (1 isolate) – KENYA, Aberdares National Park, Central Province, GPS S00°20'23.2" E036°47'11.1", tropical montane forest, altitude 2075m, waterbuck dung, 72 days incubation, 29 August 2010, Paul Mungai, KWSANP005-2010.

Notes – *Saccobolus diffusus* Sect. *Saccobolus* is morphologically very similar to *S. citrinus* but the latter has smaller apothecia, smaller ascospore clusters and smaller ornamented single ascospores (Kaushal & Viridi 1986, Doveri 2004). *S. glaber*, another similar species, is differentiated by having lemon-yellow apothecia, larger asci (140–275 × 25–48 µm), larger ascospore clusters (50–68 × 16–25 µm), larger individual ascospores (22–29 × 8.5–14.5 (–16 µm) and the presence of paraphyses-like hyphae in the upper part of the excipulum (Kaushal & Viridi 1986, Doveri 2004). This is a new record for Kenya.

***Saccobolus infestans*** (Bat. & Pontual) Brumm., *Persoonia*, Suppl. 1: 204, 1967. (Figs.8A–K)

*Ascomata* scattered or gregarious, superficial, sessile, white, margin not differentiated, 150–350 µm diam. *Ectal excipulum* of *textura globulosa*, cells 3–3.5 µm diam. *Paraphyses* simple, clavate, septate, exceeding asci, 3–4 µm broad, olive or yellow pigmented, slightly curved, slightly inflated at the tips. *Asci* 44–59 × 12–18 µm, 8-spored, unitunicate, broadly clavate, wall blueing in Melzer's reagent, operculate, with a rather short stout stipe. *Ascospores* 8–10 × 4.5–6 µm, single-celled, ellipsoidal, with broadly rounded ends, at first hyaline, finally violet to brown, verruculose or roughened with minute dots, arranged according to various forms of pattern VI of van Brummelen; clusters compact, arranged in two rows, 19–25 × 9.5–11.5 µm, cemented together by a hyaline gelatinous sheath.

Material examined (1 isolate) – KENYA, Nairobi National Park, Nairobi Province, GPS 37M0257082 9850692, altitude 1668m, grassland, zebra dung, 59 days incubation, 20 August 2010, Paul Mungai,

KWSNNP018-2010.

Notes – *S. infestans* is a unique species owing to its characteristic ascospore arrangement. This species is not likely to be confused with other currently known *Saccobolus* species (van Brummelen 1967, Doveri 2004). *S. infestans*, observed only once in a single dung type, appears to be a rare, late sporulating coprophilous fungi species from Kenyan wildlife. This is a new record for Kenya.

***Saccobolus platensis*** Gamundí & Ranalli, *Nova Hedwigia* 17: 385, 1969. (Fig. 9A–F)

*Ascomata* apothecioid, gregarious, superficial, sessile, 650–750 µm diam. *Receptacle* pulvinate, glabrous, amber to yellow, margin not differentiated. *Disc* convex or flat. *Hymenium* dotted with far protruding, almost black tips of ripe asci. *Hypothecium* composed of yellow-greyish globular cells. *Medullary excipulum* not well differentiated from *ectal excipulum*. *Ectal excipulum* of *textura globulosa* cells 5–8 × 5–8 µm. *Paraphyses* filiform, simple, septate, in yellow pigment, scanty, tips just slightly swollen, 3–4 µm diam. *Asci* 90–120 × 18–23 µm, 8-spored, unitunicate, clavate, with a pronounced apical ring, curved, usually with a fairly long stipe. *Ascospores* 14–17 × 6–9 µm, ellipsoidal, purple to brown at maturity, rough or with fine warts, truncate ends, symmetrical, arranged according to pattern I of van Brummelen; clusters compact, 35–41 × 12–16 µm, surrounded by a hyaline, common broad gelatinous sheath.

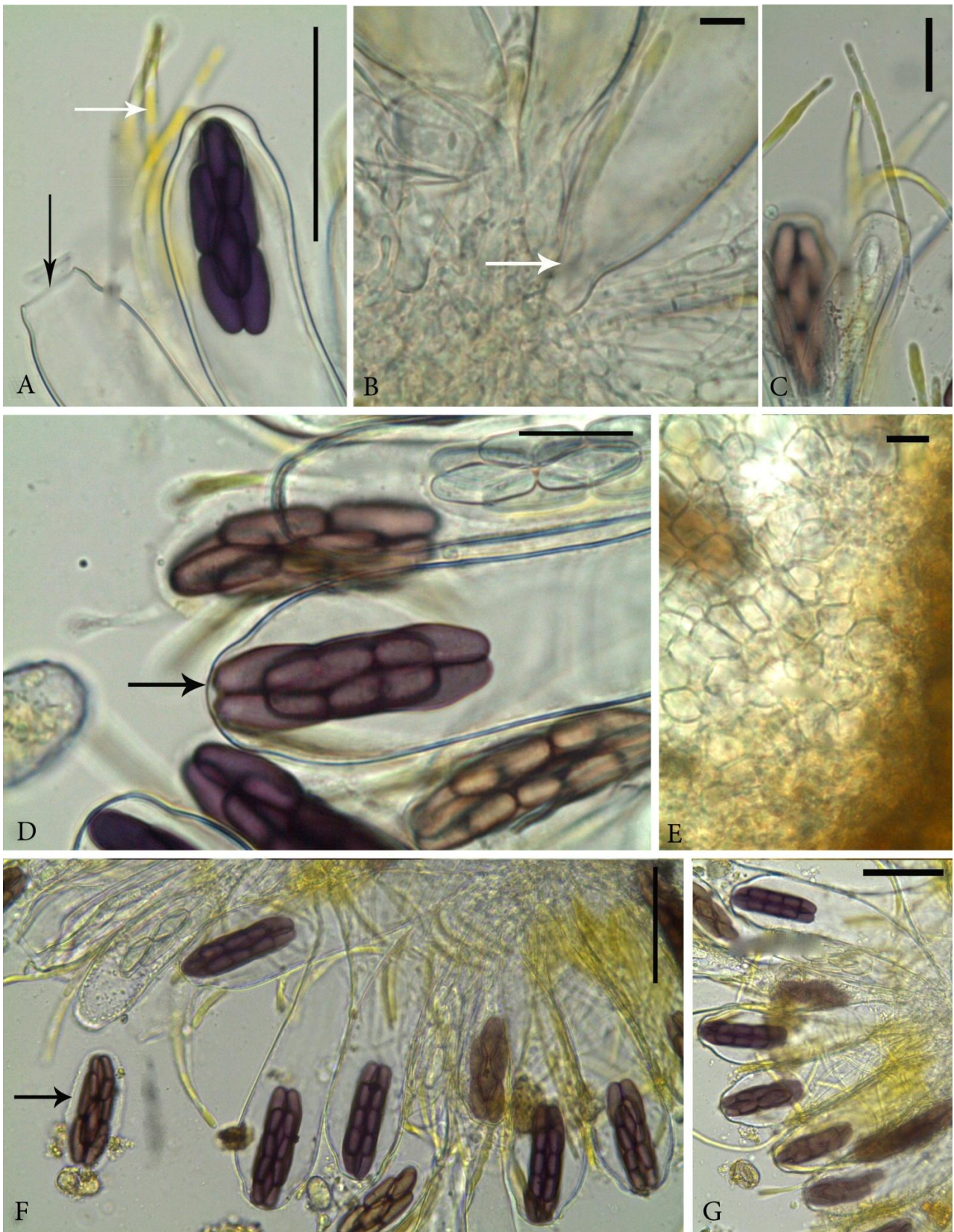
Material examined (3 isolates) – KENYA, Tsavo East National Park, Coast Province, GPS S03°21'666" E038°38'772", altitude 514m, savannah grassland, 18 and 57 days incubation, elephant dung, 23 September 2008, Paul Mungai, KWSTE005A-2008; Aberdares National Park, Central Province, GPS S00°20'23.2" E036°47'11.1", tropical montane forest, altitude 2075m, water-buck dung, 29 August 2010, Paul Mungai, KWSANP005-2010.

Notes – *Saccobolus platensis* is very similar to *S. minimus* from which it is differentiated by its smaller ascospores and asci, irregular and rough episporium (Richardson & Watling 1997, Doveri 2004). The two species share a lot of similarities and



**Fig. 6** – *Saccobolus diffusus* (KWSANP005-2009). **A** Ascomata on dung. **B** Squash ascoma. **C** Details of ectal excipulum. **D** Asci and ascospores among paraphyses. **E** Apical part of ascus. **F** Gelatinous envelope around ascospore cluster. **G** Free mature ascospores and a dehiscent ascus. **H** Ascospore arrangement, paraphyses and hyaline immature spores in yellow mucus. **I** Ascus stipe. **J** Open operculum. Scale bars: A = 500  $\mu\text{m}$ , B = 200  $\mu\text{m}$ , C = 20  $\mu\text{m}$ , D = 50  $\mu\text{m}$ , E-F = 20  $\mu\text{m}$ , G = 50  $\mu\text{m}$ , H-J = 20  $\mu\text{m}$ .





**Fig. 7** – *Saccobolus diffusus* (KWSANP005-2010). **A** Ascus showing open operculum (black arrow), apical portion and upper parts of paraphyses with yellow pigment (white arrow). **B** Ascus stipe and paraphyses, note attachment to basal cells (arrow). **C** Paraphyses. **D** Ascospores in apical portion of ascus, note closed operculum (arrow). **E** Cells of ectal excipulum. **F-G** Mature asci and ascospores, note gelatinous sheath (arrow) and the yellow pigmentation on paraphyses. Scale bars: **D-E** = 20  $\mu\text{m}$ , **F-G** = 50  $\mu\text{m}$ .



hence are hardly separable. This is a new record for Kenya.

*Saccobolus truncatus* Vel., Monogr. Discom. Boh. 1: 370, 1934. (Figs.10A–D, 11E–I)

*Ascomata* apothecioid, gregarious or solitary, sessile, 300–400 µm diam. *Receptacle* globular or lenticular, pale yellow to brownish due to ascospore colour, smooth, inconspicuous due to solitary habit and small size, margin not differentiated. *Disc* convex, yellowish, roughened by the black protruding tips of ripe asci. *Hymenium* thick. *Hypothecium* and *medullary excipulum* not well delineated from *ectal excipulum*. *Ectal excipulum* of hyaline thin walled *textura globulosa* cells. *Paraphyses* cylindrical-filiform, simple or branched, septate, exceeding the asci, 2–3 µm broad; tips slightly clavate, filled with yellow pigment. *Asci* 52–60.5 × 15–18 µm 8-spored, unitunicate, wall turning blue in Melzer's reagent, cylindrical-claviform, operculate, gradually tapering down into a stout stalk, thick walled, rounded to flattened apex. *Ascospores* 13–18 × 6–8 µm, single-celled, ellipsoidal to fusiform, sometimes slightly asymmetrical, purple to brown at maturity, finely granulate, thick walled, epispore peeling off with maturity, almost smooth, truncate ends, arranged according to pattern Ia of van Brummelen; clusters loose, 35–44 × 11.5–15 µm characteristically contracting, shortening and getting more rounded with age, with a broad hyaline gelatinous sheath.

Material examined (2 isolates) – KENYA, Tsavo East National Park, Coast Province, GPS S03°21'666" E038°38'772", altitude 514m, 54 and 57 days incubation, giraffe dung, 23 August 2008, Paul Mungai, KWSTE002A-2008; Nairobi National Park, Nairobi Province, wooded grassland, GPS S01°21'15.1" E036°46'54.1", altitude 1768m, impala dung, 31 August 2009, Paul Mungai, KWSNNP008-2009.

Notes – *Saccobolus truncatus* Sect. *Saccobolus* is related to *S. citrinus* Boud. & Torrend and *S. minimus* Velen (van Brummelen 1967, Doveri 2004). The former is differentiated from the latter by its smaller dotted ascospores and paler receptacles (van Brummelen 1967, Doveri 2004). The ascospores of *S. truncatus* contract characteris-

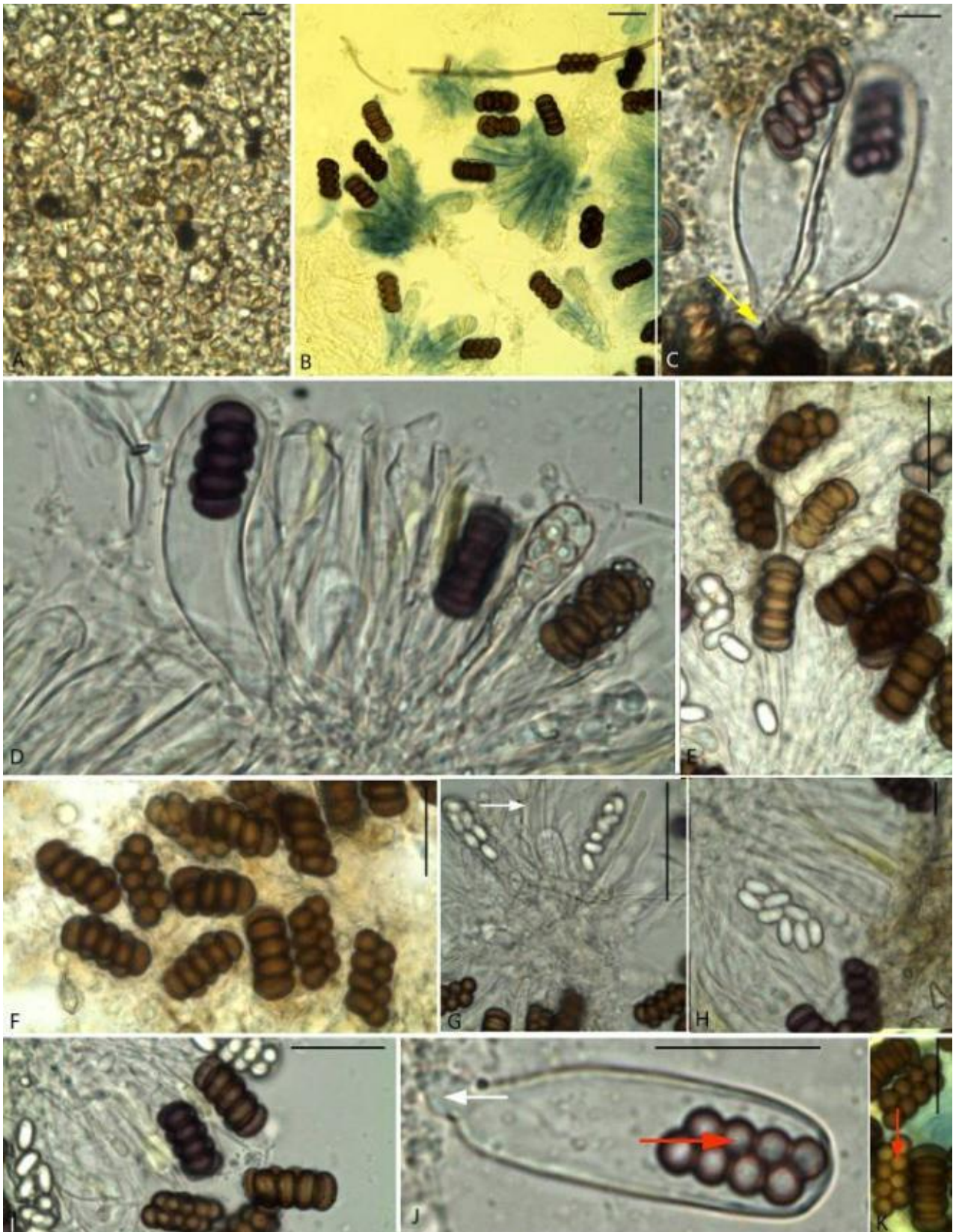
tically at maturity (van Brummelen 1967, Doveri 2004). This is a new record for Kenya.

*Saccobolus versicolor* (P. Karst.) P. Karst., Acta Soc. Fauna Flora Fenn. 2(6): 123, 1885. (Figs.12A–G, 13H–L)

*Ascomata* apothecioid, scattered to gregarious, superficial, sessile, 100–150 µm diam. *Receptacle* at first subglobose, becoming pulvinate or lenticular, external surface smooth, hyaline to violaceous or whitish grey, margin not differentiated. *Disc* convex, dark, violet or almost black tips of ripe asci dotting the hymenium, outer surface somewhat glabrous. *Hymenium* rather thick. *Hypothecium* thin, not well delineated. *Ectal excipulum* usually poorly developed of *textura-globulosa-angularis* to *intricata* elongated cells 9–16 × 3–5 µm, with very pale yellowish intercellular pigment. *Paraphyses* cylindrical-filiform, often branched and anastomosed, septate, exceeding the asci, 2.5–3.5 µm broad, hyaline; tips inflated, sometimes embedded in an intercellular violet pigment on the upper parts. *Asci* 124–146 × 30–32 µm, 8-spored, unitunicate, broadly clavate, truncate apex, walls turning blue in Melzer's reagent, operculate, short lobate stalk. *Ascospores* 15–19 × 7–9 µm, single-celled, ellipsoidal-fusiform with narrow ends, asymmetrical, purple, violaceous, becoming blackish brown with age, smooth with minute pits, sometimes with fissures, with a very thick epispore, epispore of old ascospores peeling on squashing, exposing white-creamy under-coat, arranged according to pattern II of van Brummelen; clusters compact, 41–46 × 15–18 µm broad, enclosed by a granular unilateral gelatinous envelope.

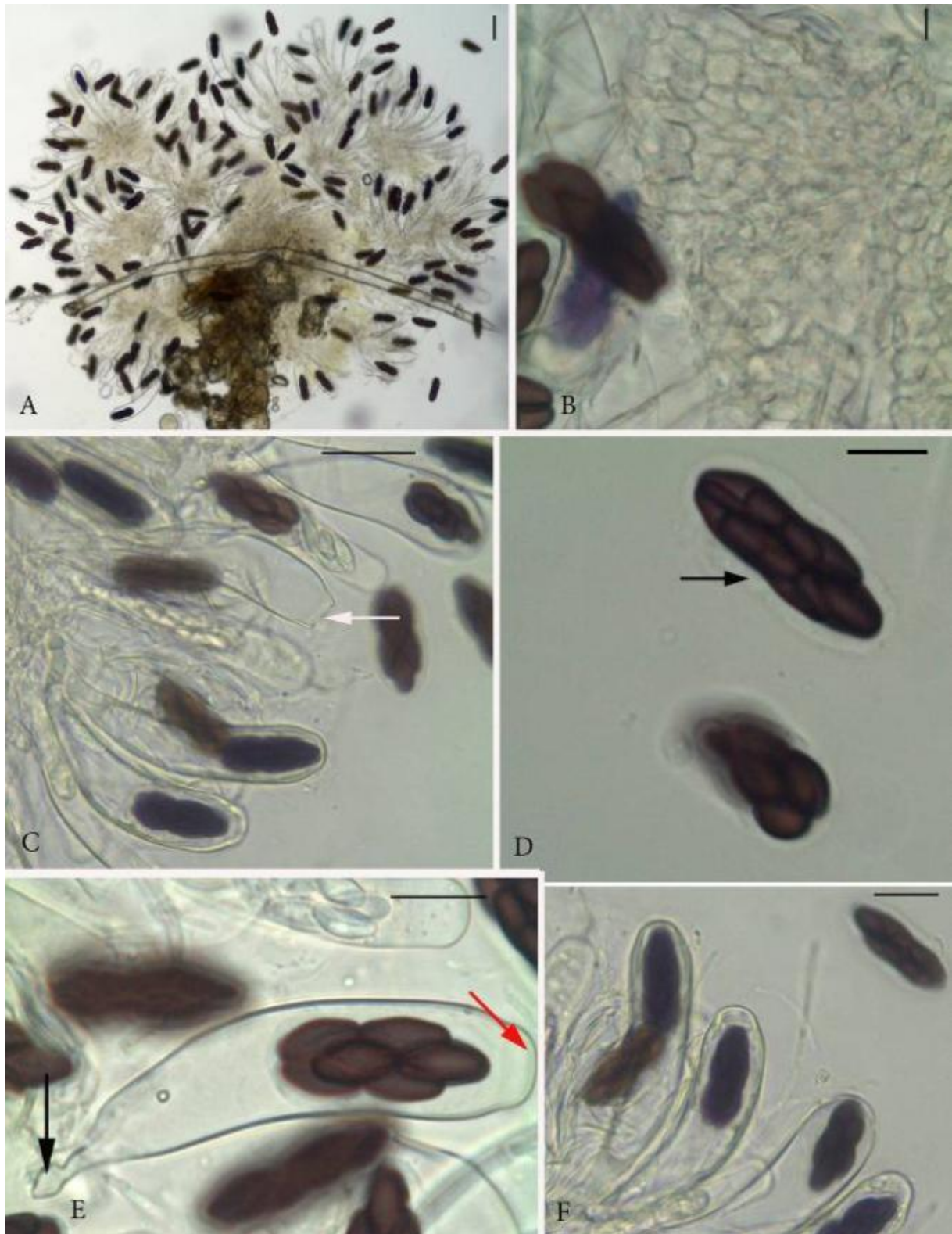
Material examined (1 isolate) – KENYA, Nairobi National Park, Nairobi Province, GPS 37M0255729 9849626, altitude 1680m, 18 days incubation, impala dung, 20 August 2010, Paul Mungai, KWSNNP020-2010.

Notes – The Kenyan collection of *Saccobolus versicolor* Sect. *Eriobolus* is notably typical with hyaline to violaceous apothecia, asci (124–146 × 30–32 µm), ascospores (15–18.5 × 7–9 µm), pigmented purple-violet, becoming rough and brown with age; clusters 41–46 × 15–18 µm (in this



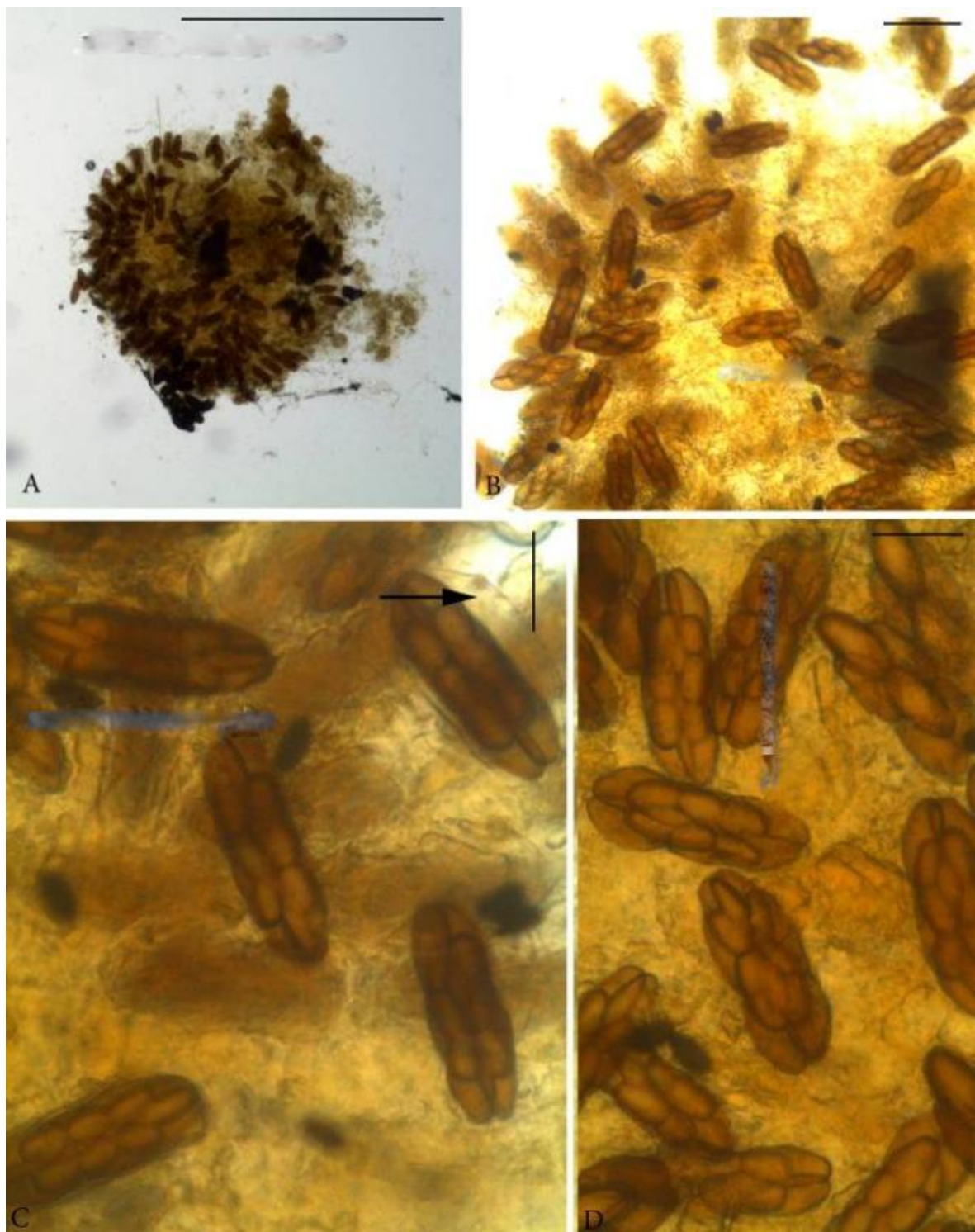
**Fig. 8** – *Saccobolus infestans* (KWSNNP018-2009). **A** Details of ectal excipulum. **B** Mature asci and ascospores. **C** Mature asci showing stipe (arrow). **D** Mature asci with spores among dehiscent asci. **E-F, I** Free mature ascospores. **G-H** Paraphyses, note colour (arrow). **J** Mature ascus with ascospores, showing a stipe and the spore arrangement (arrows). **K** Ascospores, note the arrangement (arrow). Scale bars: A = 20  $\mu$ m, B = 50  $\mu$ m, C-K = 20  $\mu$ m.





**Fig. 9** – *Saccobolus platensis* (KWSTE005A-2008). **A** Squashed ascoma. **B** Details of ectal excipulum. **C** Mature asci with ascospores, note open operculum (arrow). **D** Ascospore showing spore arrangement and gelatinous sheath (arrow). **E** Ascus, note long stipe (black arrow) and closed operculum (red arrow). **F** Asci and paraphyses. Scale bars: A = 200  $\mu$ m, B = 20  $\mu$ m, C = 50  $\mu$ m, D-E = 20  $\mu$ m, F = 50  $\mu$ m.





**Fig. 10** – *Saccobolus truncatus* (KWSTE002A-2008). **A** Squashed ascoma. **B-D** Mature ascospores, note broad hyaline gelatinous envelope (arrow). Scale bars: A = 200 µm, B = 50 µm, C-D = 20 µm.

examination). *S. versicolor* is similar to *S. caesariatus* from which it is differentiated by having hairy clusters on the external surface of its apothecia; it is also common and cosmopolitan in distribution while the latter is quite uncommon (van Brummelen 1967, Doveri 2004).

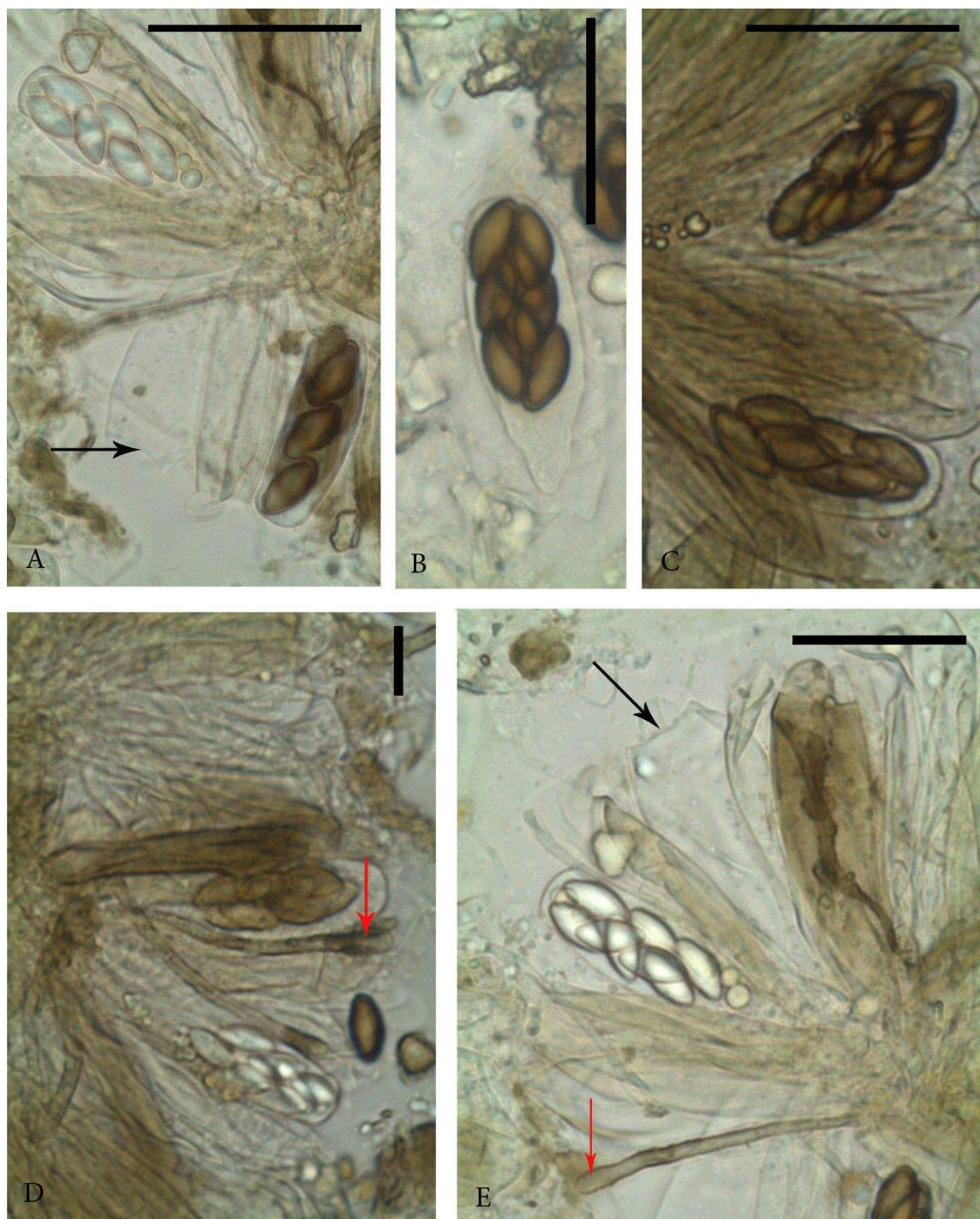
### Discussion

Our specimens are typical of *Saccobolus* in their morphology. The diagnostic features of *Saccobolus* include apothecioid ascomata, always clustered ascospores which in most cases have brown or purple-brownish episporic pigment at maturity

and with each cluster enclosed in a common gelatinous envelope (van Brummelen 1967, Doveri 2004, Bell 2005). The ascospore clusters are usually arranged in regular patterns (van Brummelen 1967). The ascus walls of *Saccobolus* are usually amyloid. The gelatinous sheath on ascospores were either unilateral or bilateral, that is surrounding the spores completely.

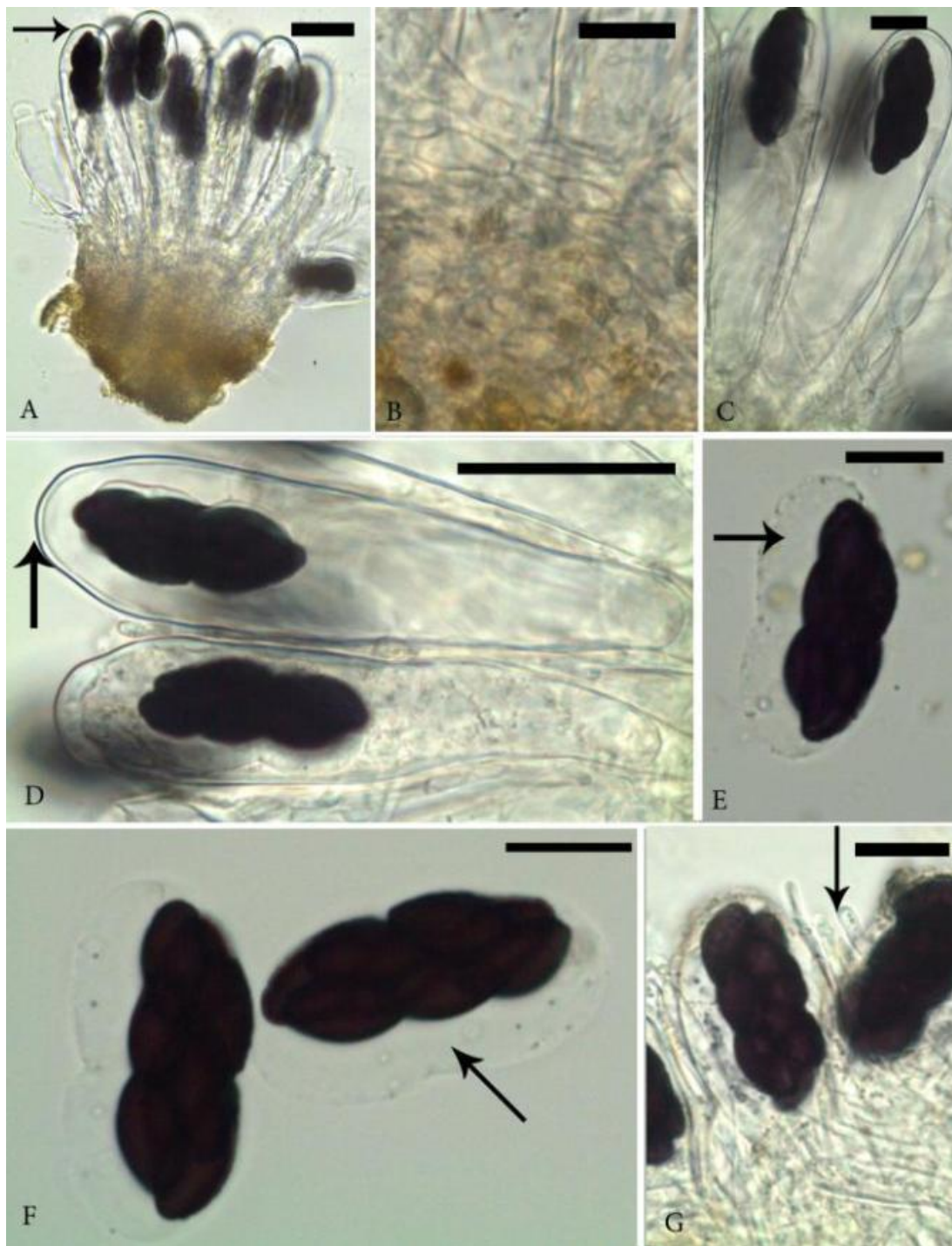
The dimensions of structures such as spores and asci are important characters when delimiting species within the genus *Saccobolus*. This study used morphological examinations to delimit the species.

New records for Kenya are *Saccobolus citrinus*, *S. diffusus*, *S. infestans*, *S. platensis* and *S. truncatus*.



**Fig. 11** – *Saccobolus truncatus* (KWSTE002A-2008). **A-C** Ascospores and asci, note open opercula (black arrow). **D** Ascii and paraphyses. **E** Ascii, note open opercula (black arrow), various stages of dehiscence and paraphyses tips (red arrows). Scale bars: **A-E** = 20  $\mu$ m.





**Fig. 12** – *Saccobolus versicolor* (KWSNNP020-2010). **A** Squash ascoma showing asci protruding above the hymenium (arrow). **B** Ascomatal wall. **C-D** Mature ascus and ascospores, note operculum (arrow). **E-F** Free mature ascospores, note gelatinous sheath (arrow). **G** Paraphyses and free ascospores (arrow). Scale bars: A = 50  $\mu$ m, B-G = 20  $\mu$ m.

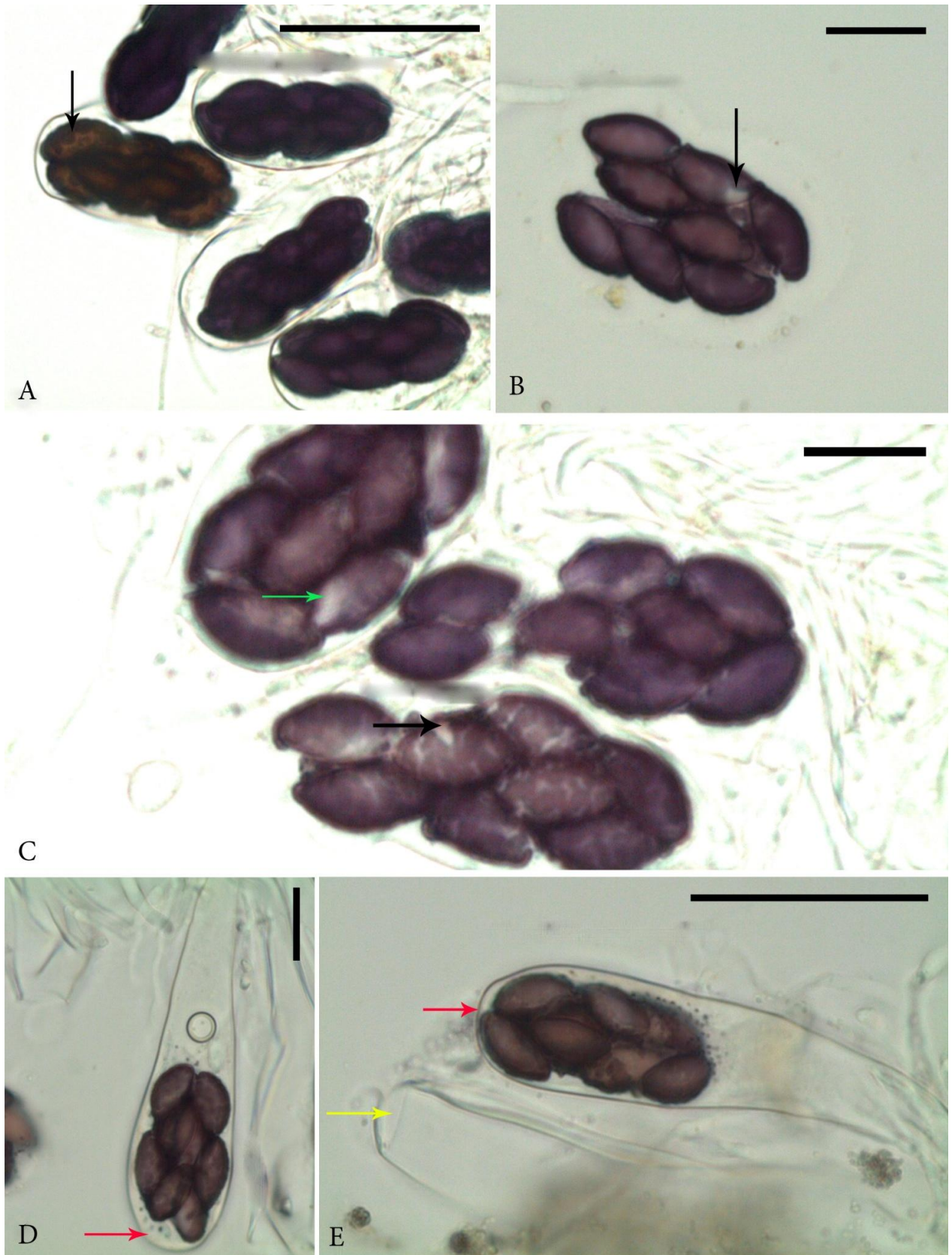
### Ecology

Twenty-four specimens of *Saccobolus* sporulated on Kenyan wildlife dung. Giraffe had 5 specimens, impala 4, waterbuck 3, African elephant 3, Cape buffalo 3, dikdik 2, zebra 2, hippopotamus 1 and hartebeest 1. *Saccobolus depauperatus* had the least substrate selectivity and sporulated on the most kinds of dung, while

*S. diffusus* and *S. infestans* were quite rare and were observed on a single dung type. Interestingly, giraffe and impala dung, a browser and a grazer, respectively, and hence totally different feeding habits, produced the most *Saccobolus* specimens.

We took note of the age of incubated dung at which *Saccobolus* species sporulated.





**Fig. 13** – *Saccobolus versicolor* (KWSNNP020-2010). **A** Ascospores in the apical portions of asci, note a portion of thicker episporium (arrow). **B-C** Ascospores squashed under cover slip, note peeling episporium (arrows). **D-E** Mature asci with ascospores, note closed and open opercula (red and yellow arrows). Scale bars: **A-E** = 20  $\mu$ m.

*Saccobolus diffusus*, *S. infestans*, *S. truncatus* and *S. versicolor* sporulated at 40 to 70 days. *S. depauperatus*, the most common species, sporulated across the entire period of dung incubation. This sequential sporulation indicated a form of succession in species of *Saccobolus*.

*Saccobolus citrinus* was found only in fresh dung from wild herbivores in Nairobi National Park.

Our results show that there is high coprophilous fungi species diversity in wildlife dung. The diversity of wild animals in Kenya also implies a corresponding high dung fungi diversity. This virtually unexplored substrate may hold numerous *Saccobolus* species currently unknown to science with yet to be recognized useful applications.

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