BIODIVERSITY AND TAXONOMY OF COPROPHILOUS ASCOMYCETES FROM WILD HERBIVORES IN KENYAN NATIONAL PARKS, EAST AFRICA

PAUL GITAU MUNGAI

MASTER OF SCIENCE
IN
BIOLOGICAL SCIENCES

SCHOOL OF SCIENCE
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THIS THESIS HAS BEEN APPROVED
TO BE A PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGICAL SCIENCES
2012

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ACKNOWLEDGEMENTS

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ABSTRACT

Coprophilous ascomycetes are saprobic fungi that have adapted to live on animal dung. Ascospores of true coprophilous ascomycetes only sporulate after passing through the gut of animals.

In this study, the diversity of coprophilous ascomycetes was investigated between 2008 and 2012. Initially, several samples of animal dung such as giraffe, zebra, elephant, dikdik, buffalo and impala were collected from Kenyan National Parks. The specimens were incubated at room temperature using the moist chamber technique for up to three months. The occurrence of dung fungi was then observed and their identity was determined based on morphological characteristics.

Based on this study, at least 10 genera of coprophilous ascomycetes were present. These included *Arnium*, *Ascobolus*, *Saccobolus*, *Chaetomium*, *Podospora*, *Schizothecium*, *Sordaria*, *Sporormiella*, *Zopfiella* and *Zygopleurage*. In total, 127 fungal isolates were examined. The most common coprophilous ascomycetes from wildlife dung were *Podospora communis*, *Sporormiella minima*, *Zygopleurage zygospora*, and *Saccobolus depauperatus*. In addition, our study also described two

new species namely, *Ascobolus nairobiensis* and *A. tsavoensis.* Seventeen new records including *Schizothecium conicum, S. dubium*, *S. curvuloides* var. *curvuloides, S. glutinans*, *Chaetomium seminis-citrulli, Sporormiella leporina, Podospora minor, Ascobolus bistisii, Ascobolus calesco, Saccobolus citrinus*, *S. diffusus*, *S. infestans*, *S. platensis, S. truncatus*, *Arnium arizonense*, *Zopfiella longicaudata* and *Sordaria fimicola* were described and illustrated from this study.

Our data revealed that abiotic and biotic factors such as wildlife species, habitat type and the season influence the occurrence, species composition, diversity and distribution of some species of coprophilous ascomycetes. We also noted that most *Ascobolus* species sporulated mainly on dung that was cultured immediately after sampling. Most *Schizothecium* species seemed to prefer dung from grazing animals. Some genera such as *Saccobolus* were observed to sporulate on several dung types, whereas others, including *Chaetomium*, were observed to prefer browser animal dung. Giraffe dung samples yielded a high diversity of coprophilous ascomycetes. The dung of impala, giraffe, dikdik, waterbuck and elephant yielded a high number of specimens and species of ascomycetes.

The animal species (dung type), dung structure, texture, moisture content and age were important variables that determined the occurrence of coprophilous ascomycetes. The age of dung at sampling and the time taken from sampling to incubation had a notable influence on the composition of ascomycetes sporulating with most of the early sporulating species being less common on old or preserved dung.

There was a predictable sequence of species sporulating indicating a form of succession in most species. However, some species were observed to sporulate across the entire period of dung incubation.

Keywords: Ascobolaceae/Diversity/Habitat/Wild animal

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CHAPTER 1

INTRODUCTION

Kenya, latitude 1°00'N and longitude 38°00'E in eastern Africa, covers a land surface of 581,677 km^2 out of which 10,749 km^2 is wetland. The equator roughly bisects the country into almost equal northern and southern parts. It has a population of 43.5 million and a population density of 75 persons per km^2 (en.wikipedia.org/wiki/Kenya, 2013). The country is placed in the second category of mega-biodiverse areas of the world (UNEP-World Conservation Monitoring Center, 1994).

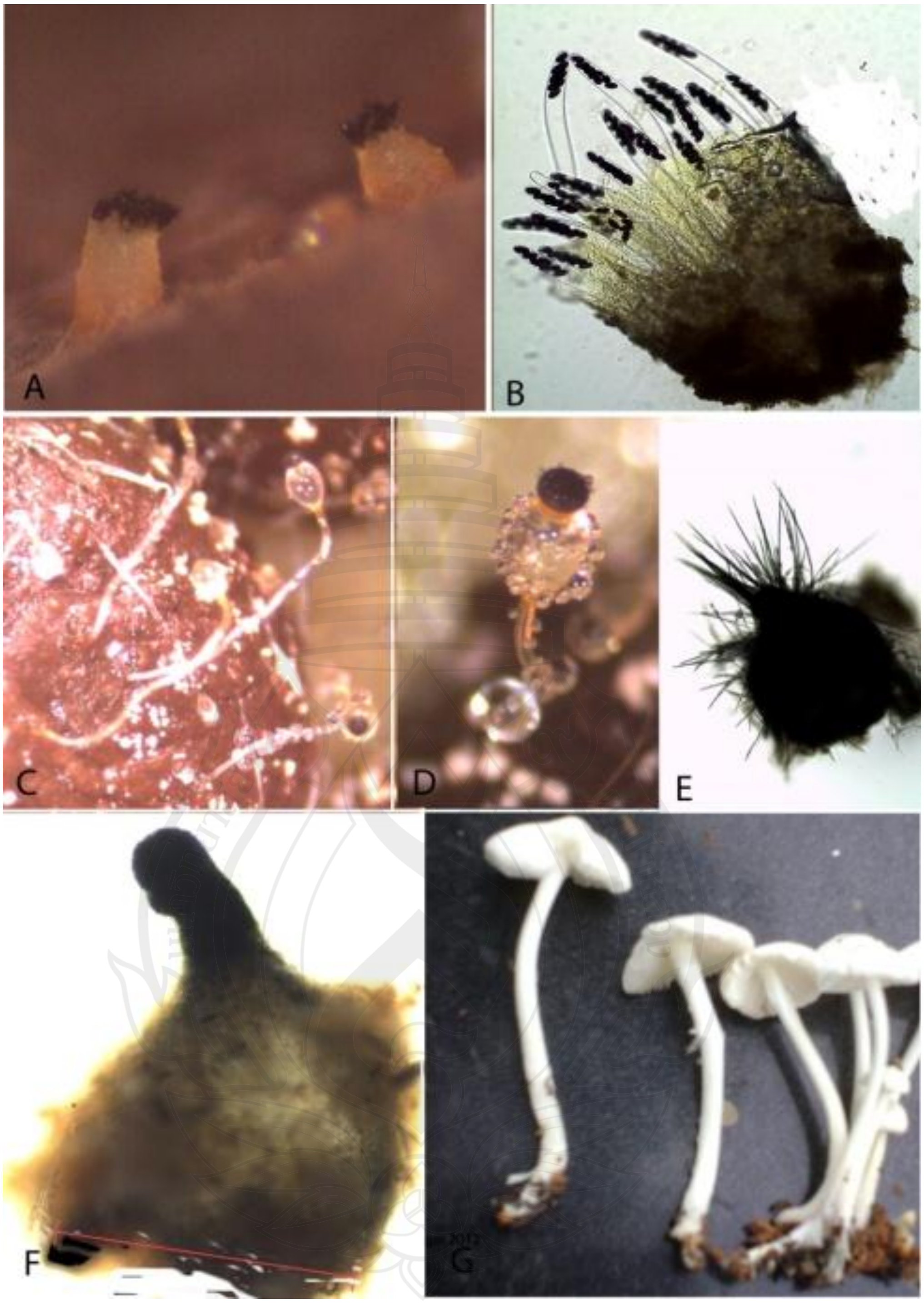
Kenya is signatory to the Convention on Biological Diversity (CBD) which recommends that member states adopt the ecosystem approach for biodiversity conservation. The ecosystem approach requires the adoption and application of appropriate scientifically sound interventions emphasizing on the levels of biological systems and essential processes, functions and interactions between organisms and their environment (CBD, 2000; Cutko, 2009). Therefore, Kenya fully applies the ecosystem approach in her biodiversity conservation and management programmes. Emphasis on biodiversity conservation and management in Kenya, according to level of importance, is mostly on flagship and endangered animals and plants. Consequently, fungi and other microbes are not considered flagship organisms and therefore do not attract much research interest.

Biodiversity conservation and management is given a very high priority as it contributes significantly to the Kenyan national economy. Kenya is renowned for her wildlife diversity and conservation and management programs that make it a global tourism destination of choice to many wildlife enthusiasts. There are over 50 protected conservation areas which occupy about 27,198 km^2 of the land mass (Kenya National Bureau of Statistics, 2009). These comprise both terrestrial and marine entities. Conservation areas are delineated in such a way that they basically represent all natural ecosystems found in Kenya ranging from mountain, coastal forests, fresh water, marine wetlands, grasslands, woodlands and semi-arid habitats in the northern part of the country. These areas are home to a wide variety of biodiversity that includes birds, invertebrates, fishes, reptiles and mammals some of which are endemics, critically or highly endangered. Though only few and isolated studies have been carried out, Kenya being in the tropics and having a wide diversity of wild animal species is naturally expected to be a coprophilous fungi species rich nation.

1.1 Coprophilous Fungi

Coprophilous fungi comprise fungi that have adapted to live on animal dung with their spores only becoming viable after passing through the digestive tract of animals (Bell, 1983, 2005; Kirk et al., 2008). This group of fungi is also referred to as dung or fimicolous fungi (Ulloa et al., 2000; Doveri, 2004; Kirk et al., 2008). True coprophilous fungi comprise of fungi specially adapted to living on dung. Their spores can only sporulate after passage through the digestive tract of animals (Bell, 1983, 2005; Kirk et al., 2008). Some naturally occurring biochemicals present in the digestive tract of animals act on the spores "pre-treating" and making them viable. Coprophilous fungi are made up of both obligate and facultative species. Obligate species strictly sporulate on dung under natural conditions while facultative species are opportunistic and can grow on other substrates as well. The diversity of coprophilous fungi is closely related to the occurrence and distribution of the animal hosts on which they depend on. Though the actual diversity of coprophilous fungi is largely unknown, they have been shown to be ubiquitous (Lundqvist, 1972; Bell, 1983, 2005).

Coprophilous fungi have representatives from all the known classes of fungi namely, ascomycetes, basidiomycetes, myxomycetes, zygomycetes and asexual fungi (Figure 1.1 and Table 1.1).



Note. (A-B) Ascomata of *Ascobolus* an apothecial ascomycetes. (C-D) Zygosporangia of *Pilobolus* a zygomycetes. (E-F) Ascomata of *Podospora* a perithecial ascomycetes (G) Basidiomata of *termitomyces* a basidiomycetes.

Figure 1.1 Macroscopic Features of Some Coprophilous Fungi.

Table 1.1 Representative Genera of Fungi Commonly Isolated from Dung

Fungal group	Genus
Ascomycetes	*Ascobolus* Pers.
	Cercophora Fuckel
	Chaetomium Kunze
	Coprotus Korf & Kimbr.
	Iodophanus Korf
	Lasiobolus Sacc.
	Podospora Ces
	Saccobolus Boud.
	Schizothecium Corda
	Sporormiella Ellis & Everh.
	Zopfiella G. Winter
	Zygopleurage Boedijn
Basidiomycetes	*Agrocybe* Fayod
	Bolbitius Fr.
	Conocybe Fayod
	Coprinus Pers.
	Panaeolus (Fr.) Quél.
	Psilocybe (Fr.) P. Kumm.
	Sebacina Tul. & C. Tul.
Zygomycetes	*Cunninghamella* Matr.
	Mucor P. Micheli ex Fr.
	Pilaira Tiegh.
	Pilobolus Tode.
	Rhizopus Ehrenb.
Myxomycetes	*Arcyria* Hill ex F.H. Wigg.
	Badhamia Berk.
	Dictyostelium Bref.
	Metatrichia Ing
	Physarum Pers.
	Trichia Haller
Anamorphic fungi	*Acremonium* Link
	Aspergillus P. Micheli
(asexual form)	*Botrytis* P. Micheli ex Pers.
	Cladosporium Link
	Fusarium Link
	Geotrichum Link
	Graphium Corda
	Oedocephalum Preuss.
	Penicillium Link
	Phialocephala W.B. Kendr.
	Phialophora Medlar
	Scopulariopsis Bainier

Adopted from Kendrick (2002)

Fungal taxonomy is a dynamic and progressive discipline that regularly demands changes in nomenclature. A challenge in fungi taxonomy is that traditionally, fungi are mostly classified on the basis of their appearance as opposed to bacteria in which the nutritional and biochemical differences play a very significant role in classification (Guarro et al., 1999). Unlike animals and plants reproductive compatibility is not very useful in fungal taxonomy considering that they can be both sexual and asexual.

As a result of these challenges, different concepts have been applied in fungal taxonomy. To define a species in fungi, the first concept is the morphological (phenotypic) concept, a classic approach used where units are defined not only on the basis of morphological characteristics but also by the differences among them (Nannfeldt, 1932; Lundqvist, 1972; Barr, 1983; Bell, 1983, 2005; Berbee & Taylor, 1992; Guarro et al., 1999; Webster & Weber, 2007). However, even where intact, fresh and mature reproductive structures are available, this system still has limitations. The second is the phylogenetic concept where molecular techniques are employed to examine and analyze the DNA or indirectly analyze the properties of fungi proteins such as enzymes (Webster & Weber, 2007).

Most coprophilous fungi especially the ascomycetes are generally phototrophic and as such they discharge their spores towards light (Bell, 2005). Phototropism of the asci determines the direction to which the spores are released. The head of the ascus is usually pushed above the surface of the apothecium or through the ostiole of the perithecium (Brummelen van, 1967; Bell, 1983, 2005; Doveri, 2004). The ascus is directed towards light where spores are shot to during release (Brummelen van, 1967). This ensures that spores are dispersed away from the fruiting body onto surrounding herbage from where they are ingested by a grazing animal to perpetuate the cycle (Lundqvist, 1972; Bell, 1983, 2005). The non-phototrophic coprophilous fungi species disperse their ascospores through other means such as wind, water or animals (Kendrick, 2002).

Coprophilous fungi have a close relationship with their herbivorous hosts (Lundqvist, 1972). Some specificity between fungi-host on one hand and environment-fungi on the other hand has been observed (Lundqvist, 1972; Richardson, 1972). Many coprophilous ascomycetes are ubiquitous, while others show a marked preference for a particular dung type (Lundqvist, 1972), for example, *Sporormiella minimoides* S.I.

Ahmed & Cain is mostly seen on carnivore dung while others such as *Sporormiella minima* (Auersw.) Ahmed & Cain are generalists (Ahmed & Cain, 1972; Doveri, 2004). Dung from closely related herbivores generally show somewhat similar species composition (Richardson, 2001). Sheep, a ruminant and hare, a lagomorph both feeding on the same vegetation displayed marked differences in species compositions attributable to differences in digestive system (Richardson, 2005). This implies that the digestive system of the herbivore may influence species composition and richness, as the differences in digestive system and processes could affect both the passage of the spores through the gut, dung moisture, length of time spores spend inside the gut, type of gut biochemicals and nutrient content.

Differences in ascomycetes species richness and composition may also reflect differences in feeding habits and/or food choice between herbivores (Ebersohn & Eicker, 1997). According to Lundqvist (1972), the reason(s) for any specificity is unclear but Kruys & Ericson (2008) argue that variable food intake and choice, habitat, type of digestive system and plant species foraged influence coprophilous ascomycetes species composition diversity. Coprophilous fungi are also found on dung of non-herbivorous animals including carnivores, omnivores, birds, invertebrates and reptiles (Krug et al., 2004).

1.2 Coprophilous Ascomycetes

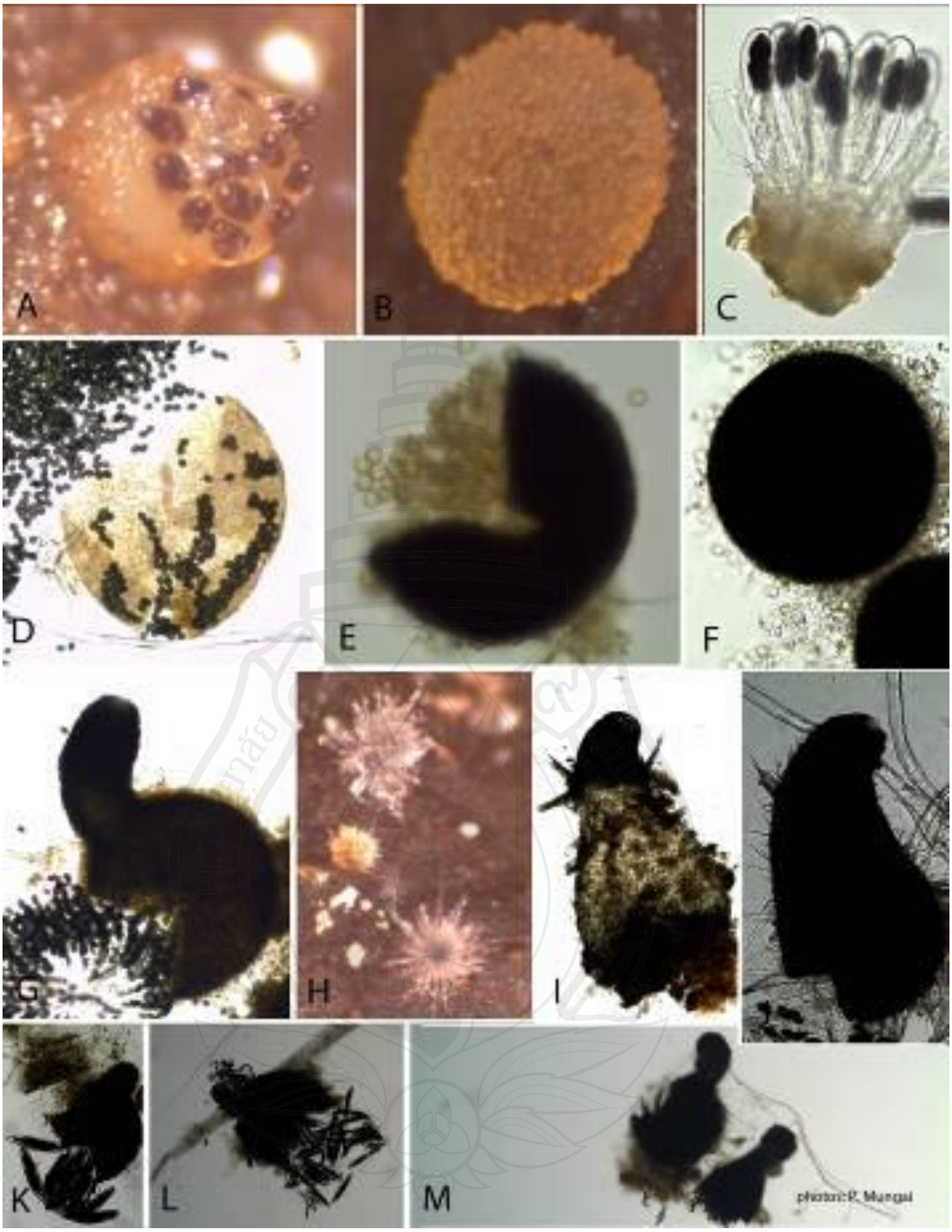
Large quantities of dung are continuously being produced by an equally large number of animal species in national parks and reserves. Although produced as "waste matter" resulting from an animal's digestive process, this offers an unrivalled micro-habitat for a wide range of living organisms that include mycobiota (Lundqvist, 1972; Rowland, 1975; Bell, 1983). According to Kendrick (2002) about 175 genera of known ascomycetes are coprophilous. The remains of plant and animal material plus the microbiota associated with digestive systems of host animals render dung a very rich medium for fungal growth. The material contains large quantities of carbohydrates, cellulose, hemicellulose and lignin. It may also include some fatty acids, vitamins, amino acids and also digestive by-products that are high in nitrogen such as keratin from hairs, skin and hooves in dung of carnivores or omnivores (Bell, 1983; Kendrick, 2002). On voiding, the feacal material is at almost a neutral pH, in varying degrees of wetness, consistence and moisture holding capacity (Richardson, 1972; Bell, 1983, 2005). Coprophilous ascomycetes are able to utilize complex feacal biomass constituents such as cellulose, hemicelluloses, keratin and lignin (Kendrick, 2002).

Ascomycetes found growing on dung may include those that have passed through an animal's gut and other saprophytes that land after deposition. These usually exist as mycelia, ascomata or ascospores (Rowland, 1975; Bell, 1983; Kendrick, 2002; Kirk et al., 2008). Several methods of ascospores dispersal are evident for the various groups of coprophilous ascomycetes. These methods range from blastic spore expulsion facilitated by phototropism, such as those of *Ascobolus* spp, *Saccobolus* spp and *Podospora* spp, to wind, rain and animal dispersal in *Chaetomium* spp and *Sporormiella* spp (Ingold, 1965; Kendrick, 2002; Bell, 2005). Insects play an important role in fungi spore dispersal, a fact overlooked by many scientists (Stevenson & Dindal, 1987). The head of the ascus is usually pushed above the surface of the apothecium or through the ostiole of the perithecium. Adhesive ascospores are actively discharged onto adjacent vegetation and then consumed by herbivores, surviving passage through the animal gut, germinate and grow as fruiting bodies (ascomata) on freshly deposited dung to complete the cycle (Lundqvist, 1972; Rowland, 1975; Bell, 1983, 2005; Kendrick, 2002). Non-adhesive species also find their way into the guts of animals through various means, survive passage through the animal gut, germinate and grow as fruiting bodies in a cycle similar to their adhesive counterparts. According to Fischer et al. (2004) and Yafetto et al. (2008) ascospores of blastic species can be shot off to about 2.5 metres away, clearly a fascinating and remarkable natural phenomenon. Methods of dispersal, reproduction and growth in ascomycetes offer insights into a very unique and complex life cycle.

Coprophilous ascomycetes exist as either obligate or facultative coprophilous (Ulloa et al., 2000; Doveri, 2004; Kirk et al., 2008). Obligate coprophilous ascomycetes comprise fungi strictly and specially adapted to living on dung which only sporulate after their spores have passed through the digestive tract of animals (Bell, 1983, 2005; Kirk et al., 2008). Facultative coprophilous fungi, such as *Chaetomium* spp, on the other hand are opportunistic and grow on other substrates as well. Certain genera have a high proportion of coprophilous representatives with some such as *Ascobolus*, *Podospora* and *Sporormiella* being more or less exclusively coprophilous (Bell, 1983). At present the actual global diversity of coprophilous ascomycetes is not known, however, it is believed they are ubiquitous and cosmopolitan. This group of fungi is also found on dung of non-herbivorous animals including carnivores, omnivores, birds, invertebrates and reptiles (Lundqvist, 1972; Krug et al., 2004). Herbivore dung is easy to work with compared to carnivore dung which becomes quite obnoxious after a few days incubation. It is not yet clear how arboreal animals such as monkeys and birds get spores of dung fungi yet their food is largely high up on trees above the ground.

Like other ascomycetes, the basic diagnostic character of coprophilous ascomycetes is the production of spores in a sac-like structure known as the ascus. The major groupings of coprophilous ascomycetes, as recognized in early classification, were based on the type of ascomatal development (Lundqvist, 1972; Barr, 1983; Bell, 1983, 2005; Alexopolous et al., 1996; Webster & Weber, 2007). In the scheme, ascomycetes were grouped into the classes Plectomycetes, characterized by closed ascomata, Discomycetes, with disc-like ascomata and lastly Pyrenomycetes bearing flask-shaped ascomata (Bell, 1983). Present day classification, however, though still largely based on these for general descriptive purposes is further elucidated by molecular techniques and phylogenetic studies (Suh & Blackwell, 1999; Bell, 2005).

In the classification scheme, coprophilous ascomycetes are classified based on fruiting body type, either as cleistothecial, perithecial, apothecial or pseudothecial (Bell, 1983, 2005; Kendrick, 2002, see Figure 1.2). Cleistothecial ascomycetes have closed ascoma; perithecial ascomycetes are recognized by having an ascoma that opens by a narrow orifice, while in apothecial ascomycetes the ascoma opens broadly like a cup while the pseudothecial ascoma is a form in between perithecial and cleistothecial (Bell, 1983, 2005; Kendrick, 2002; Webster & Weber, 2007; Kirk et al., 2008).



Note. (A-C) apothecial ascomata. (D-F) cleistothecial ascomata. (G-J) perithecial ascomata. (K-M) pseudothecial ascomata.

Figure 1.2 Coprophilous Ascomycetes Fruiting Body Types.

In phenotypic classification, ascomatal characters come in handy. For instance, the broad grouping mentioned in the paragraph above has been done using the presence or absence of an ostiole. Of great importance in classifying the ascoma is the habit, whether it is immersed, semi-immersed, erumpent, superficial and/or sessile. Other characters such as the type of ascocarp (apothecial, perithecial, cleistothecial or pseudothecia), shape of ascocarp (globose, subglobose or ellipsoidal) are also important. Variables of size, that is height and width, neck, shape, size, colour, hairy, smooth are noted, the peridial structure either *angularis, globularis, prismatica, intricata* or a combination of these; ascocarp coverings: hairy / spiny / smooth or furfuraceous; ascocarp contents: paraphyses / pseudoparaphyses / epiphyses / cellular help in species diagnosis. Ascocarp colour and form of hymenium: flat/convex/concave. These characters are very important in classification, for instance, on account of its agglutinated hairs *Schizothecium* is differentiated from the closely related *Podospora* (Lundqvist, 1972; Bell & Mahoney, 1995; Bell, 1983, 2005; Doveri, 2008). Some genera such as *Lasiobolus* and *Cheilymenia* are differentiated by the absence or presence of septa on their ascomatal hairs (Denison, 1964; Moravec, 2005; Bell, 2005).

Ascus characters are very useful in circumscribing genera and species. Important details include the shape which can be: cylindrical / ellipsoidal / clavate / globose / subglobose; number: numerous / countable / others; size: length / width; wall: chemical reactions with Melzer's / Congo red / lactophenol blue; apex: rounded / flat / truncate; stipe: with / without / length / width singly or jointly can help identify a species or genus. For instance genera with bitunicate ascus wall are separated from those with unitunicate wall. For example, ascomycetes with bitunicate walled asci (double-layered) belong to a group classified as loculoascomycetes (Barr, 1987, 1997, 2000). Further, Pezizaceae and Ascobolaceae are differentiated from other Pezizales by having asci that usually turn blue in Melzer's reagent (Brummelen van, 1967; Korf, 1972; Bell, 1983, 2005; Kendrick, 2002; Doveri, 2008). The number of spores per ascus, the shape, size of the ascus and stipe are also important diagnostic characters and can be useful in differentiating species such as within *Sporormiella* (Korf, 1972; Doveri, 2004; Bell, 2005). The mode of ascus dehiscence is also an important character.

Ascospore characters are used extensively in genera and species delimitation. These include, spore shape classified as: globose / ellipsoidal / subglobose / reniform or limoniform, the formation and septation, occurring singly / cluster or bundle and with or without septa. The arrangement inside the ascus: uniseriate / biseriate / multiseriate or irregularly placed; spore size: length / width; pigmentation: hyaline or coloured. It is important to note the presence or absence of a gelatinous sheath/envelope: unilateral / bilateral / broad or narrow; germ pore: with or without; perispore: smooth / rough / ornamented or guttulate; the presence or absence and shape of a germ slit on ascospores is useful in delimiting species in Sporormiaceae (Barr, 1979, 1987, 2000; Doveri, 2004; Bell, 2005).

The characters of paraphyses are also critical in diagnosis. These include details of shape: clavate / filiform / jacket or other forms; size: length and width; tips: swollen / pointed / curved or other forms; septa: with or without; pigmentation: with or without; vacuoles: with or without; anastomosing: seen or not seen; paraphyses branching: present or absent. It has been shown that pigments produced in the paraphyses play an important role in diagnosis of species and groups of ascomycetes. For instance, *Saccobolus* section *Saccobolus*, with yellowish amber fruit bodies, is differentiated from *Saccobolus* section *Eriobolus*, with white, violet or brownish fruit bodies. (Brummelen van, 1967). Branching, tips and anastomosing of paraphyses are useful in circumscribing species (Korf, 1972). Jacket-like paraphyses are a feature used to differentiate *Schizothecium* from the closely related *Podospora* (Bell & Mahoney, 1995; Doveri, 2008).

1.3 Life Cycle of Coprophilous Ascomycetes

Ascomycetes are either single-celled (yeasts) or filamentous (hyphal) or both (dimorphic) (Bell, 1983; Kendrick, 2002; Webster & Weber, 2007). Yeasts grow by budding or fission while hyphae grow apically and branch laterally (Kendrick, 2002; Webster & Weber, 2007). Most yeasts and filamentous ascomycetes are haploid, but some species, for example *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, can also be diploid with a double set of chromosomes (Kendrick, 2002; Webster & Weber, 2007). According to Alexopoulos et al. (1996) mitospores may simply reproduce the parent or may also act as gametes to fertilize a compatible partner. Some ascomycetes referred to as heterothallic must outbreed, others can self-fertilize as well, while still some known as homothallic can only self-fertilize (Figure 1.3).

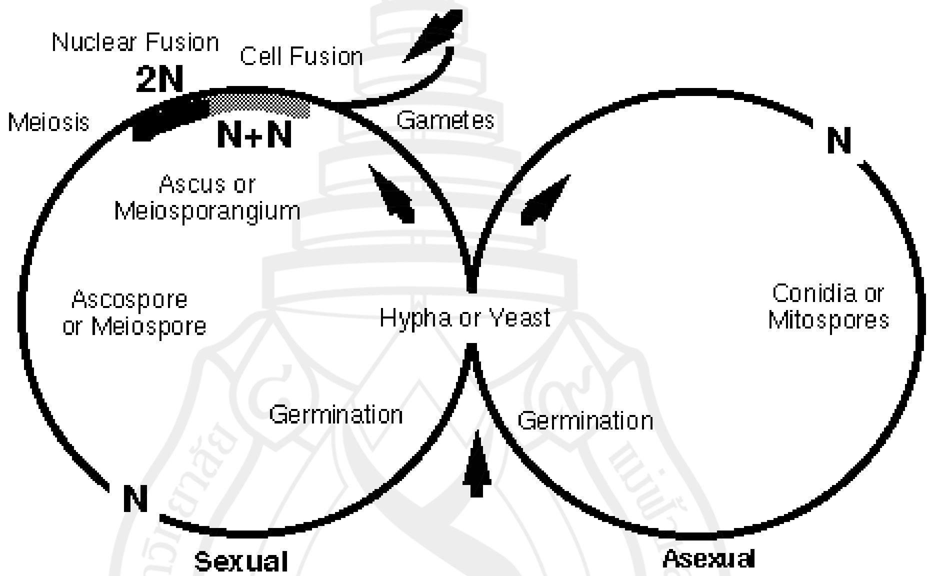


Figure 1.3 Life cycle of Ascomycetes (Taylor,1996)

Genetic control of sex expression and mating has been studied well in some model ascomycetes, such as yeasts that have two sexes and where mating is coordinated by oligopeptide pheromones, (Marsh, 1991; Glass & Lorimer, 1991). In hyphal species, cytoplasmic fusion may not be immediately followed by nuclear fusion, leading to a short dikaryotic phase (Webster & Weber, 2007). The dikaryotic hyphae may be protected and nourished by differentiated haploid hyphae which form fruiting bodies (ascomata) (Webster & Weber, 2007). Ascospores that are released from an ascoma germinate to form new haploid mycelia. From a human perspective, a fascinating and most unusual phenomenon of all fungi is that they have more than one reproductive option (Kendrick, 2002; Webster & Weber, 2007).

Upon release from their dung-inhabiting fruiting bodies, spores of many dung fungi through various ways end up falling onto the surrounding herbage from where they are swallowed again by foraging animals thereby perpetuating a cycle (Figure 1.4). Feeding and foraging animals swallow large amounts of fungal spores hence the presence of many dung fungi spores in their droppings (Bell, 1983, 2005). To survive during passage through the gut of animals, many species of dung ascomycetes have spores with thick walls. These walls weaken due to digestive action and thus prepare the spores for sporulation once they have been deposited with the animal's droppings (Bell, 1983, 2005). Herbivore dung is usually composed of plant and other materials in varying degrees of digestion and breakdown. At the time of voiding a number of dung ascomycetes may have spores ready to germinate (Bell, 1983, 2005). Many germinate mostly at the same time but their mycelia grow at varying rates. It is believed that the sequence of fruiting body appearances reflects the speed of mycelial growth and the time it takes for a mycelium to accumulate enough resources to allow the production of fruiting bodies (Rayner & Todd, 1979; Hyde & Jones, 2002; Richardson, 2002).

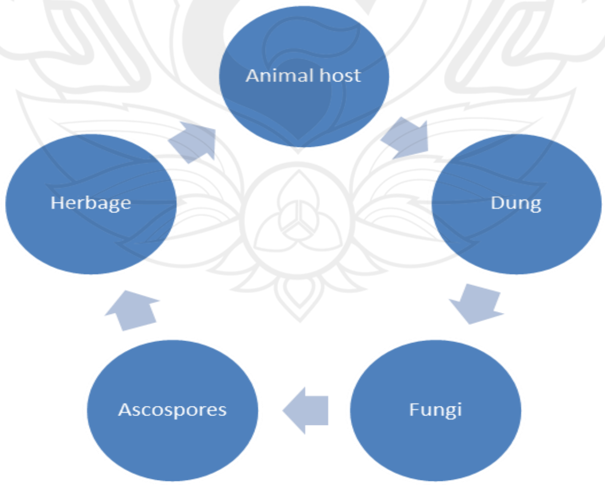


Figure 1.4 Ecology of Coprophilous Ascomycetes

1.4 Coprophilous Fungi Succession

Over the years, the phenomenon of fungal succession has attracted the interest of many investigators who have tried to understand this interesting phenomenon (Harper & Webster, 1964; Bell, 1975; Rayner & Todd, 1979; Safar et al., 1988; Richardson, 2001). From these investigations, various theories as to what and how fungi succession happens have been postulated ranging from the time taken by fruit body to mature, incubation temperature to the effects of bio-chemical composition of dung (Wicklow et al., 1980; Pardo, Sivori & Ranalli, 1997; Magnelli & Forchiasini, 1999). Harper & Webster (1964) point to that fact the apparent correlation between the decomposition of progressively complex carbon sources and the taxonomic disposition of these species and competition between and within species could also play a role in succession.

Fungi succession has been defined "as the sequential occupation of the same site by thalli (usually mycelia) either of different fungi or of different associations of fungi" by Harper & Webster (1964) and Rayner & Todd (1979) both of whom emphasize that succession is the sequential occupation of a site by mycelium of different species of fungi in a predictable manner.

The subject of fungal succession has recently been reviewed by Frankland (1992, 1998), Jones & Hyde (2002), Suzuki (2002), Hyde & Jones (2002), Fryar (2002) and Richardson (2002) who suggest that fungal succession may as well be termed "mycelial succession" to account for the importance of observable changes in mycelial occurrence and distribution. However, it is generally agreed that a true succession of fungi sporulating on dung in essence means both the mycelia, invisible but inside the dung, and the visible fruiting bodies and their sequential colonization of the dung (Harper & Webster, 1964; Webster, 1970; Rayner & Todd, 1979; Richardson, 2001, 2002; Hyde & Jones, 2002; Jones & Hyde, 2002; Richardson, 2002). In view of this, Fryar (2002) recommends that the term "mycelia succession" should be used in order to avoid confusion and emphasize the changes that occur in the distribution of mycelia during this process.

In fungal succession, zygomycetes (*Pilaira* / *Pilobolus* / *Mucor*) always appear first, followed by ascomycetes (*Lasiobolus* / *Iodophanus* / *Ascobolus* / *Podospora* / *Sporormiella* / *Schizothecium*). Last to appear are the basidiomycetes (*Coprinus* / *Psilocybe* / *Conocybe* / *Agrocybe* / *Bolbitius* / *Coprinopsis / Coprinellus* / *Cyathus* / *Panaeolus*) (Harper & Webster, 1964; Wicklow et al., 1980; Richardson, 2001, 2002).

Richardson (2002) in a study on coprophilous fungi was able to empirically demonstrate that each of the main taxonomic groups of coprophilous fungi follow a definite succession pattern, for instance, ascomycetes also sporulate in a predictable pattern. He observed the following pattern: *Lasiobolus* Sacc. was the first to sporulate, followed by *Thelebolus* Tode, *Ascozonus* (Renny) E.C. Hansen, *Saccobolus* Boud., *Sporormiella* Ellis & Everh., *Sordaria* Ces. & De Not., *Schizothecium* Corda, *Ascobolus* Pers., *Delitschia* Auersw., *Podospora* Ces. Section *Rhyophila*, *Schizothecium*, *Podospora* excluding Sect. *Rhyophila*, *Iodophanus* Korf and *Arnium* Nitschke ex G. Winter with *Coprotus* Korf & Kimbr. sporulating in the last period. This study gives a clearer synopsis of this process.

Factors influencing and driving succession are constant and similar in all the regions studied both in the northern and southern hemispheres (Dade, 1957; Harper & Webster, 1964; Mitchell, 1970; Larsen, 1971; Richardson, 1972; Rowland, 1975; Bell, 1975; Nagy & Harrower, 1979; Kuthubutheen & Webster, 1986; Ebersohn & Eicker, 1997; McCarthy, 2000; Richardson, 2002).

Though maturing slowly, some dung fungi are very antagonistic to other species. They are able to destroy and severely inhibit the development of mycelia of other species (Bell, 1983). This characteristic has generated a lot of interest to mycologists interested in biotechnology research and development (R&D) of natural products (Kendrick, 2002). In addition to fungi, various bacteria, nematodes, mites and arthropods also inhabit dung (Rowland, 1975; Bell, 1983, 2005). Therefore dung can be viewed as a complex substratum and forms an ecological microcosm between different organisms and their environment which thus influences the appearance of fungi fruiting bodies (Rowland, 1975).

Understanding the succession phenomenon using phenotypic species characterization has notable inherent limitations. The presence of a mosaic of species on the dung microcosm poses a challenge in unequivocal species diagnosis. As observed earlier, fungal species exist in both asexual and sexual states, thus further aggravating taxa discrimination. The DNA based-biosystematics techniques are a promising approach to improving knowledge about taxonomy, succession and function of coprophilous fungal communities (Berbee & Taylor, 1992; Koschinsky et al., 1999; Liu et al., 1997; Cai et al., 2005; Chang & Wang, 2010). Molecular techniques constitute an important complement to the morphological criteria needed to enable fungi to be more easily and accurately identified beyond the fruiting body (Lee & Taylor, 1992; Berbee & Taylor, 1992; Guarro et al., 1999; Kendrick, 2002; Webster & Weber, 2007). DNA extracted directly from coprophilous fungi hyphae and mycelia can be used as template for PCR amplification and detection of specific fungi without the need for culturing (Liu et al., 1997; Koschinsky et al., 1999; Rodriguez et al., 2004, Jones et al., 2011; Liu et al., 2011; Yang, 2011). Therefore, relatively accurate, fast and reliable fungi monitoring can be done using molecular techniques. For example, DNA fragments are generated through restriction fragment length polymorphism (RFLP) or random amplified polymorphic DNA (RAPD). Molecular techniques can be enhanced through the use of target sequences encoding the 18S or 28S ribosomal RNA (rRNA) molecules or the non-coding DNA stretches (internal transcribed spacer: ITS) which physically separate these genes from each other (Huhndorf et al., 2004; Cai et al., 2005; Chang & Wang, 2010). The crucial role of the molecular technique to coprophilous fungi classification was demonstrated by Cai et al. (2005), using ribosomal DNA and protein coding genes, where it was possible to conclusively resolve the long running argument on the classification of *Podospora* and *Schizothecium* clearly showing that the two are indeed different but closely related genera. In another study, Kruys & Wedin (2009), through a molecular phylogenetic examination of Sporormiacae using combined data sets of ITS-nLSU rDNA, mtSSU rDNA and ß-tubulin, were able to clarify the phylogenetic relationships, test current generic classification and the utility of phenotypic characters traditionally used in generic classification in this group. Chang & Wang (2010) examining Lasiosphaeraceae using ribosomal DNA and a fragment of Glyceraldehyde-3- phosphate dehydrogenase (GPD) were able to shed light on the classification and relationships of *Schizothecium*, *Podospora* and *Cercophora* hitherto confusing taxonomy. A host of methods have recently been developed for community analysis (Koschinsky et al., 1999; Liu et al., 2011; Jones et al., 2011; Halme et al., 2012). These include denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (tRFLP) and single-strand-conformation polymorphism (SSCP). These can greatly facilitate accurate monitoring of coprophilous fungal succession. By way of these methods, it may be possible to identify coprophilous fungi by analyzing their respective mycelia/hyphae and fruit bodies as they grow and develop thus help in elucidating the fungi succession processes more elaborately.

In the developed world, citizen or amateur monitoring of fungi biodiversity contributes a lot of data on fungi distribution and occurrence. This helps to complement the work being done by the few mycologists in those regions. The application of molecular techniques offer quicker and more precise classification but it is unfortunately quite a complicated procedure and can only be used by scientists thus excluding the participation of this important source of fungal information. However molecular techniques, while used together with the classical phenotypic techniques, offer a very accurate classification method for all groups of fungi including anamorphs (Kendrick, 2002). Phylogenetic species criteria will result in faster recognition of many more species than those delimited by phenotypic criteria alone thereby leading to much higher coprophilous fungi species diversity.

1.5 Role and Significance

Biodiversity in national parks is a major tourist attraction and contributes significantly to the national economy. The ecological integrity and health of the national parks is important to the survival of the thousands of wild animals which live there. Fungi play major ecological roles which in turn influence the well-being of ecosystems. Classification and documentation of the fungal diversity in the national parks will help identify indicator fungi species which in turn may be used to monitor the health of these conservation areas. If fully documented, the fascinating world of coprophilous ascomycetes may as well be an added attraction to science-oriented tourists. Some coprophilous ascomycetes such as *Sporormiella* have been used in palaecological studies such as predicting the occurrence and distribution of mega faunal species (Robinson et al., 2005).

Most coprophilous ascomycetes are remarkably prolific and relatively easy to culture and thus offer an unrivalled pool of useful organisms. Many among them have been observed to produce antibiotic substances that inhibit the growth of competitors (Bell, 1983, 2005). As shown in Table 1.2, one of their most fascinating properties is the capacity to synthesize secondary metabolites that display a wide range of bioactivity (Fox & Howlett, 2008). Most mycologists believe that high-value products useful in enhancing mankind's quality of life, such as applications in ecological rehabilitation and restoration, habitat preservation, agriculture, biomass recycling, bioremediation of industrial wastes manufacture of dyes, veterinary medicine, biofuel, genomics and genetic engineering will be discovered from fungi (Kendrick, 2002; Obire et al., 2008).

Table 1.2 Some Bioactive Metabolites Isolated From Coprophilous Fungi

Bioactive compounds	**Applications/activity**	**Fungal species**	**References**
Zaragozic acid A, B and C, (squalestatins)	Cholesterol lowering (cholesterol biosynthesis inhibitors) and Alzheimer's Disease (AD) treatment via alpha-secretase activation	*Sporormiella intermedia* (Auersw.) S.I. Ahmed & Cain ex Kobayasi and *Leptodontium elatius* (F. Mangenot) de Hoog	Bergström et al. (1992); Kojro et al. (2010)
Terezines A-D	Antifungal	*Sporormiella teretispora* S.I. Ahmed & Cain	Wang et al. (1995)
Naphthopyranone	Selectively phytotoxic (herbicidal) weed killer	*Guanomyces polythrix* M.C. González, Hanlin & Ulloa	Macias et al. (2001)
Communiols E-H (Polyketides)	Anti-fungal and antibacterial	*Podospora communis* (Speg.) Niessl	Yongshen et al. (2005)
Pseudodestruxins A and B	Anti-fungal and antibacterial	*Nigrosabulum globosum* Malloch & Cain	Yongshen et al. (2001)
Sporostatin	Anti-fungal and antibiotic	*Sporormiella* sp.	Murakami et al. (1999)
Preussomerins G to L	Anti-cancer, Antiplasimodial, Anti-bacterial and antifungal	*Preussia isomera* Cain	Krohn et al. (2001)
Sordarins	Anti-yeast	*Podospora pleiospora* (G. Winter) Niessl	Weber et al. (2005)
Antiamoebins I, III, XVI and myrocin B	Anti-amoeba	*Stilbella erythrocephala* (syn. *S. fimetaria*) (Ditmar) Lindau	Lehr et al. (2006)
Curvicollides A-C	Polyketide	*Podospora curvicolla* (G. Winter) Niessl	Yongshen et al. (2004)
Sporminarins A and B	Anti-fungal polyketides	*Sporormiella minimoides* S.I. Ahmed & Cain	Mudur et al. (2006)
Aflaquinolones	Anti-fungal and insecticidal	*Aspergillus* sp.	Neff et al. (2012)
Polytolypin	Anti-fungal	*Polytolypa hystricis* J.A. Scott & Malloch	Gamble et al. (1995)
Psylocibin	Psychiatric and palliative uses	*Psylocibe* sp.	Grob et al. (2011)
Cyclosporine	Immuno-suppressant	*Tolypocladium niveum* (O. Rostr.)Bissett	Kendrick (2002)

1.6 Coprophilous Fungi in Kenya

Numerous studies on the ecology of coprophilous ascomycetes have been conducted in the temperate regions of the world. However, the tropical and the warm arid regions have attracted relatively fewer investigators. Countries in Africa, South America and Asia have not done well in this field (Table 1.3). The earliest documented fungal studies in East Africa were carried out by Berkley (1885), Hennings (1893), Duke (1926) and Hanford (1941).

In a majority of the coprophilous fungi studies, there has been a bias towards dung of a limited number of domesticated, semi-domesticated and a few wild animals (Wicklow et al., 1980; Angel & Wicklow, 1983; Elshafie, 2005; Masunga et al., 2006; Jeamjitt et al., 2007). Wildlife dung as a substrate has been much less studied.

Studies on the occurrence and distribution of coprophilous fungi in Kenya are relatively quite recent and not extensive. Some noteworthy initiatives covered the entire east African region (Kenya, Uganda and Tanzania). These include works on coprophilous Sordariaceae namely *Podospora*, *Sporormiella* and *Chaetomium* (Khan & Cain, 1972; Khan & Cain, 1979; Carter & Khan, 1982). Other surveys include Minoura (1969), Krug & Khan (1987, 1989), Khan & Krug (1989a, 1989b) and Caretta et al., (1998). Surveys for the rest of the African region include Morocco, Egypt, Libya, South Africa and the Central African Republic (Cailleux, 1969, 1971; Rattan & El-Buni, 1979, 1980; Ebersohn & Eicker, 1992; Richardson, 2004b; Abdel-Azeem, 2004, 2009; N' Douba et al., 2010). These surveys mainly examine ascomycetes sporulating on herbivore dung.

Kenya is renowned for her diverse ecosystems and remarkable wild animal species diversity both invertebrates and vertebrates living in equally diverse habitats. Studies have shown that coprophilous fungi diversity is correlated to animal and habitat diversity (Lundqvist, 1972; Bell, 1983, 2005; Kruys & Ericson, 2008). In view of this, the potential for this country to harbour many undescribed species of coprophilous fungi is high. The results of the fungi surveys done in Kenya compare very closely with studies done in other tropical regions of the world such as Asia (Wang, 1999), Australia (Bell, 2005) and South America (Watling & Richardson, 2010). These studies have shown that coprophilous ascomycetes species richness and diversity decreases with increasing latitude (Richardson 2001).

This work is, therefore, an attempt to investigate the diversity of coprophilous ascomycetes in Kenya especially on the less investigated wildlife dung substrate. Several dung samples collected from conservation areas in Kenya were used to screen and isolate coprophilous ascomycetes. The results obtained are expected to provide detailed information relevant to understanding the diversity of this important fungal group to enable its inclusion in biodiversity conservation and management programmes.

1.7 Objectives

The objectives of this study were to:

1.7.1 describe and characterize coprophilous ascomycetes species and diversity in selected Kenyan National Parks and Reserves

1.7.2 develop a checklist of Kenyan wildlife coprophilous ascomycetes and

1.7.3 create awareness on the importance of coprophilous fungi in biodiversity conservation and management.

Table 1.3 Diversity of Coprophilous Fungi from Selected Countries in Northern and Southern Hemisphere

Country	No. of samples	Dung types	Total genera	Total species	Ascomycetes	Basidiomycetes	Zygomycetes	Anamorphs	References
Brazil	7	5	14	32	22	8	2	-	Richardson (2001)
Czech Republic	23	3	-	50	26	2	10	12	Vanova & Kubatova (2004)
Egypt	180	3	55	101	33	2	16	50	Abdel-Azeem (2005, 2009)
Iceland	32	4	25	81	66	12	3	-	Richardson (2004a)
India	28	28	22	33	8	1	7	16	Rohin et al. (1973)
Iraq	60	3	19	40	34	2	3	1	Abdullah (1982)
Italy	60	1	53	82	18	1	7	25	Piontelli et al. (1981)
Italy	60	3	-	364	219	20	54	71	Caretta et al. (1994)
Italy	722	-	-	348	256	92	-	-	Doveri (2011)
Japan	220	-	-	52	-	-	-	-	Furuya & Udagawa (1972)
Kenya	15	12	40	59	23	1	5	30	Caretta et al. (1998)
Morocco	14	5		57	50	5	2	-	Richardson (2004b)
Puerto Rico and Antilles	21	6	19	52	37	9	6	-	Richardson (2008)
South Africa	-	4	32	96	21	2	5	17	Ebersohn & Eicker (1992)
Thailand	56	4	16	20	20	-	-	-	Jeamjitt et al. (2007)
Thailand	60	13	57	69	27	1	7	28	Jeamjitt (2007)
UK	137	6	20	51	51	-	-	-	Richardson (1972)

CHAPTER 2

INVENTORY OF KENYAN COPROPHILOUS ASCOMYCETES

2.1 Introduction

To undertake sound and sustainable conservation of fungi, data on habitat, occurrence, population size, species abundance and distribution and interactions with the environment is very crucial (Janzen & Hallwachs, 1994). Kenya has elaborate wildlife conservation and management programmes whose primary objective is to conserve and manage a select group of animals considered charismatic or ecological keystone species. Unfortunately, less charismatic life forms have not attracted similar interest from conservation scientists and managers. Indeed, the conservation of some rare or imperiled species in less charismatic organisms is dependent upon the conservation of associated and co-occurring charismatic species (Cutko, 2009).

Fungal biodiversity, more so the state of coprophilous ascomycetes remains largely unexplored in Kenya. Some studies have been conducted on coprophilous fungi in Kenya (Caretta et al., 1998; Minoura, 1969; Gatumbi & Kungu, 1994; Khan & Krug, 1989a; Khan & Krug, 1989b; Krug & Cain, 1974; Krug & Khan, 1989; Khan & Cain, 1972; Lundqvist, 1973; Lundqvist, 1981). The coprophilous fungal surveys conducted show a high diversity of coprophilous mycobiota in Kenya (Table 2.1).

This work is intended to form a baseline for future dung fungal surveys and expand on the checklists developed in these surveys.

Table 2.1 Known Coprophilous Fungi in Kenya

Fungal species	Dung type/Substrate	Location	Reference
cremoniella verrucosa Tognini	reedbuck, steenbok, zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Acremonium sp.	steenbok	Marula Estates, Naivasha	Caretta et al.(1998)
Acremonium strictum W. Gams	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Actinomucor elegans (Eidam) Benjamin & Hesseltine	reedbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Alternaria alternata (Fr.) Keissler	reedbuck, bushbuck, waterbuck, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Arthrobotrys oligospora Fres.	reedbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Ascobolus americanus (Cke. et Ellis) Seaver	antelope	Ngong Road Forest	Minoura (1969)
Ascobolus immersus Pers.	reedbuck, steenbok, impala, waterbuck, buffalo, zebra, dikdik	Marula Estates, Naivasha	Caretta et al.(1998)
Ascobolus perplexans Massee & Salmon	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Ascobolus sp.	dikdik	Marula Estates, Naivasha	Caretta et al.(1998)
Ascobolus viridulis Phill. et Plow.	cow	Ngong Road Forest	Minoura (1969)
Ascodesmis nigricans van Tieghen	antelope	Ngong Road Forest	Minoura (1969)
Ascophanus lacteus (Cke. et Phill.) Sacc.	carnivore	Ngong Road Forest	Minoura (1969)
Bipolaris cynodontis (Marignoni) Shoemaker	impala, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Camarosporium sp.	reedbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Chaetomium bostrychodes Zopf	reedbuck, steenbok, impala, bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Chaetomium convolutum Chivers	bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Chaetomium crispatum (Fuckel)	soil,wheat straw	Nairobi KARI labs	Gatumbi & Kungu (1994)
Chaetomium fusiformes Chivers	antelope	Ngong Road Forest	Minoura (1969)
Chaetomium globosum Kunze ex Fr.	antelope	Ngong Road Forest	Minoura (1969)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Chaetomium murorum Corda	soil, wheat straw, waterbuck, zebu	Nairobi KARI labs Marula Estates, Naivasha	Gatumbi & Kungu (1994) Caretta et al. (1998)
Chaetomium olivaceum (Cooke & Ellis)	soil, wheat straw	Nairobi, KARI labs	Gatumbi & Kungu (1994)
Chaetomium subspirales Chivers	antelope	Ngong Road Forest	Minoura 1969
Chaetomium undulatum Bainer	soil, wheat straw	Nairobi, KARI labs	Gatumbi & Kungu (1994)
Cladorrhinum foecundissimum Sacc. & Marchal	impala, waterbuck, dikdik	Marula Estates, Naivasha	Caretta et al. (1998)
Cladosporium cladosporioides (Fresen.) de Vries	bushbuck, waterbuck, hippo, buffalo	Marula Estates, Naivasha	Caretta et al.(1998)
Cladosporium sp.	bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Coprinus sp.	steenbok, impala, bushbuck, zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Coprotus disculus Kimbr. Luck-Allen & Cain	impala	Marula Estates, Naivasha	Caretta et al.(1998)
Coprotus niveus (Fuckel) Kimbr.	impala, waterbuck, hippo, buffalo, dikdik,	Marula Estates, Naivasha	Caretta et al.(1998)
Curvularia lunata (Wakker) Boedjn	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Dactylaria sp.	bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Doratomyces columnaris Swart	zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Doratomyces stemonitis (Pers.:Fr.) Morton & G. Smith	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Endophragmiella dimorphospora (Awao & Udagawa) S.J. Hughes	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Epicoccum purpurascens Ehrenb	hippopotamus, buffalo	Marula Estates, Naivasha	Caretta et al.(1998)
Fusarium chlamydosporium Wollenw. & Reinking	reedbuck, impala, bushbuck, hippopotamus, zebra	Marula Estates, Naivasha	Caretta et al.(1998)
Fusarium sp.	reedbuck, steenbok, waterbuck, zebra	Marula Estates, Naivasha	Caretta et al.(1998)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Gelasinospora brevispora R.S. Khan & J.C. Krug	cow, zebra	Voi, Shimba Hills, Amboseli, L. Amboseli	Khan & Krug (1989a)
Gelasinospora dictyophora R.S. Khan & J.C. Krug	antelope, cow	Aberdares Ranges, Kajiado	Khan & Krug (1989a)
Gelasinospora hapsidophora R.S. Khan & J.C. Krug	elephant	Mt Kenya NP	Khan & Krug (1989a)
Gliocladium roseum Bainier	reedbuck, steenbok, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Iodophanus cameus (Pers.) Korf	steenbok, impala, bushbuck, waterbuck, buffalo, zebra, dikdik, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Kernia nitida (Sacc.) Nieuwland	reedbuck, waterbuck, dikdik, zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Lasiobolus lasioboloides Marchal	steenbok, impala, ushbuck,waterbuck, hippopotamus, zebra, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Melanospora zamiae Corda	steenbok, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Metarhizium anisopliae (Metschn.) Sorok	steenbok, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Microsphaeropsis olivacea (Bonord.) Hohn.	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Mucor hiemalis Wehmer	zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Mucor sp.	buffalo	Marula Estates, Naivasha	Caretta et al.(1998)
Myrothecium roridum Tode ex Steudel	bushbuck, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Myrothecium verrucaria (Albertini & Schwein.) Ditm. ex Steudel	steenbok	Marula Estates, Naivasha	Caretta et al.(1998)
Oedocephalum glomerulosum (Buill.) Sacc.	reedbuck, steenbok, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Phialophora sp. (anamorph of *Podospora conica*)	bushbuck, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Pilobolus crystallinus (Wiggers) Tode	reedbuck, steenbok, impala, bushbuck, waterbuck, hippopotamus, buffalo, zebu, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Pilobolus sp.	impala, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Podosordaria phoenicia	zebra	N/K	Krug & Cain (1974)
Podospora aloides (Fuckel) Mirza & Cain	buffalo	Mt Kenya NP	Krug & Khan (1989)
Podospora anserina (Ces. ex Rab.) Niessl.	zebra, cow, reedbuck, steenbok,	Nairobi NP, L. Amboseli	Krug & Khan (1989)
[*Podospora pauciseta* (Ces.) Traverso]	impala, bushbuck, waterbuck, hippopotamus, buffalo, zebra, dikdik, zebu, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora argentinensis (Speg.) Mirza & Cain	cow	Mt Kenya NP	Krug & Khan (1989)
	zebra	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora australis (Speg.) Niessl.	zebra, giraffe, cow steenbok, impala, bushbuck	Nairobi NP, Kajiado, L. Amboseli	Krug & Khan (1989)
	hippopotamus, buffalo, zebra, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora austro-americana (Speg.) Mirza & Cain	rock hyrax, carnivore	Mt Kenya NP, Machakos	Krug & Khan (1989)
Podospora caligata R.S. Khan & Cain	antelope, cow	Nairobi NP, Mt Kenya NP	Krug & Khan (1989)
	cow	Kajiado	Khan & Cain (1972)
Podospora comata Milovtzova	reedbuck, steenbok, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora communis (Speg.) Niessl.	zebra, buffalo, wildebeest, cow, elephant	Aberdare Ranges, Nairobi NP, Mt Kenya NP, Kajiado, Voi, Amboseli	Krug & Khan (1989)
Podospora conica (Fuckel) Bell & Mahoney	impala, bushbuck, hippopotamus, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora curvicolla (Winter) Niessl	duiker	n/k	Lundqvist (1973)
Podospora curvispora (Cain) Cain	elephant, cow, rock hyrax	Mt Kenya NP	Krug & Khan (1989)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Podospora curvuloides Cain	cow	Mt Kenya NP	Krug & Khan (1989)
	n/k	n/k	Lundqvist (1973)
Podospora decipiens (Winter) Niessl	buffalo, cow	Aberdares Ranges, Mt Kenya NP, Kajiado	Krug & Khan (1989)
Podospora deropodalis R.S. Khan & Cain	cow, zebra	Kajiado, Mombasa,	Krug & Khan (1989)
	zebra	Amboseli, L. Amboseli	Khan & Cain (1972)
Podospora fimicola Ces.	buffalo, eland	Mt Kenya NP	Krug & Khan (1989)
Podospora fimiseda Ces.	antelope	L .Amboseli	Krug & Khan (1989)
Podospora hyalopilosa (Stratton) Cain	antelope, cow, zebra, buffalo	Nairobi NP, Kajiado, Machakos, Mtito Andei, Amboseli, L. Amboseli	Krug & Khan (1989)
Podospora longicaudata (Griff.) Cain	cow, antelope, buffalo	Kajiado, Voi, Mombasa, L. Amboseli	Krug & Khan (1989)
Podospora millespora (A. Schimdt) Cain	antelope	Ngong Road Forest	Minoura (1969)
Podospora multispora R.S. Khan & Cain	wildebeest	Nairobi NP	Krug & Khan (1989)
	n/k	n/k	Khan & Cain (1972)
	cow	Kajiado	Khan & Cain (1972)
Podospora ostilingospora Cain	cow	Machakos	Krug & Khan (1989)
Podospora papillata Khan & Cain	n/k, zebra, elephant	N/K, L. Amboseli	Khan & Cain (1972)
Podospora perplexans (Cain) Cain	buffalo	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora platensis (Speg.) Niessl.	cow, elephant	Voi	Krug & Khan (1989)
Podospora pleiospora (Winter) Niessl.	elephant, buffalo	Aberdare, Mt Kenya NPs	Krug & Khan (1989)
Podospora prethopodalis Cain	zebra, unknown herbivore, antelope, elephant, rabbit	Nairobi NP, Amboseli, L. Amboseli	Krug & Khan (1989)
	n/k	n/k	Lundqvist (1973, 1981)

Table 2.1 (Continues)

Fungal species	Dung type/Substrate	Location	Reference
Podospora setosa (Winter) Niessl.	bushbuck, zebra, giraffe, antelope, cow steenbok, bushbuck	Aberdare Ranges, Nairobi NP, Mt Kenya NP	Krug & Khan (1989)
	antelope	Marula Estates, Naivasha	Caretta et al.(1998)
		Ngong Road Forest	Minoura (1969)
Podospora similis (Hansen) Niessl	zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora spinulosa Khan & Cain	giraffe, cow, zebra, buffalo cow, elephant	Nairobi NP, Mt Kenya NP, Mtito Andei, Mombasa, L.	Krug & Khan (1989)
		Amboseli, L. Amboseli	Khan & Cain (1972)
Podospora tetraspora (Winter) Cain	rock hyrax	Mt Kenya NP	Krug & Khan (1989)
Ramichloridium apiculatum (Miller, Giddens & Foster) de Hoog	hippopotamus, buffalo, zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Saccobolus depauperatus (Berk. et Br.) Phill.	antelope	Ngong Road Forest	Minoura (1969)
Saccobolus versicolor (P. Karst.) P. Karst.	reedbuck, steenbok, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Saccobolus violascens Boud.	antelope	Ngong Road Forest	Minoura (1969)
Sepedonium niveum Massee & Salmon	steenbok	Marula Estates, Naivasha	Caretta et al.(1998)
Sordaria alcina Lundq.	zebra	Aberdares Ranges	Khan & Krug (1989b)
Sordaria brevicollis L.S. Olive & Fantini	zebra, buffalo	Mtito Andei, L. Amboseli	Khan & Krug (1989b)
Sordaria fimicola (Rob.) Ces. & De Not.	antelope, elephant, buffalo, eland, rock hyrax, cow, zebra, steenbok	Aberdare Ranges, Aberdare N.P., Nairobi NP, Mt Kenya NP, Machakos, Mtito Andei, Mombasa, Voi, Amboseli,	Khan & Krug (1989b)
		L. Amboseli	Caretta et al.(1998)
		Marula Estates, Naivasha	
Sordaria humana (Fuckel) Winter	buffalo goat and antelope	L. Amboseli	Khan & Krug (1989b)
		Ngong Road Forest	Minoura (1969)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Sordaria lappae Poteb.	antelope, wildebeest, elephant, buffalo, cow, eland, rock hyrax, zebra	Aberdare N.P., Aberdare Ranges, Nairobi NP, Mt Kenya NP, Amboseli, L. Amboseli	Khan & Krug (1989b)
Sordaria superba De Not.	bushbuck, wildebeest, elephant	Aberdare Ranges, Nairobi NP, Mt Kenya NP	Khan & Krug (1989b)
Sordaria tomento-alba Cailleux	antelope, bushbuck, elephant, zebra	Aberdare Ranges, Mt Kenya NP, Mtito Andei, Amboseli	Khan & Krug (1989b)
Sporomiella minima (Auersw.) Ahmed & Cain	reedbuck, steenbok, impala, bushbuck, waterbuck, hippopotamus, buffalo, zebra, dikdik, zebu, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Sporormiella australis (Speg.) Ahmed & Cain	rock hyrax, eland, elephant, antelope, zebra, cow	Aberdare Ranges, Nairobi NP, Mt Kenya NP	Khan & Cain (1979)
Sporormiella capybarae (Speg.) Ahmed & Cain	cow, buffalo	L. Amboseli	Khan & Cain (1979)
Sporormiella dubia Ahmed & Cain	giraffe, buffalo, zebra, cow	Nairobi NP, Mt Kenya NP, Amboseli, L. Amboseli	Khan & Cain (1979)
Sporormia fimetaria De Not.	cow, antelope, zebra, buffalo	Kajiado, Voi, Mombasa, Amboseli, L. Amboseli	Khan & Cain (1979)
Sporormia fimicola S.I. Ahmed & Asad	cow	L. Amboseli	Khan & Cain (1979)
Sporormiella herculea (Ell. & Ev.) Ahmed & Cain	cow	L. Amboseli	Khan & Cain (1979)
Sporormiella intermedia (Auersw.) Ahmed & Cain	antelope, rock hyrax, zebra, buffalo	Nairobi NP, Mt Kenya NP, Amboseli	Khan & Cain (1979)
Sporormiella isomera Ahmed et Cain	antelope, elephant, carnivore, zebra antelope	Nairobi NP, Mt Kenya NP, Mtito Andei, L. Amboseli, Ngong Road Forest	Khan & Cain (1979) Minoura (1969)
Sporormiella kansensis (Griff.) Ahmed & Cain	antelope	L. Amboseli	Khan & Cain (1979)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Sporormiella longispora (Cain) Ahmed & Cain	giraffe	Nairobi NP	Khan & Cain (1979)
Sporormiella longisporosis Ahmed & Cain	zebra	Amboseli	Khan & Cain (1979)
Sporormiella macropulchella Khan & Cain	cow	L. Amboseli, Mt Kenya NP	Khan & Cain (1979)
Sporormiella megalospora (Auersw.) Ahmed & Cain	rock hyrax	Mt Kenya NP	Khan & Cain (1979)
Sporormiella minima (Auersw.) Ahmed & Cain	buffalo, duiker, antelope, elephant, zebra, cow, rock hyrax	Aberdare Ranges, Nairobi &, Mt Kenya NPs, Mtito Andei, Kajiado, Voi, Machakos, Mombasa, L. Amboseli	Khan & Cain (1979)
Sporormiella minimoides Ahmed & Cain	unidentified herbivore	Nairobi NP	Khan & Cain (1979)
Sporormiella muskokensis Ahmed & Cain	rock hyrax	Mt Kenya NP	Khan & Cain (1979)
Sporormiella obliqua Khan & Cain	cow, antelope, wildebeest, rabbit	L. Amboseli, Mt Kenya NP	Khan & Cain (1979)
Sporormiella pilosa (Cain) Ahmed & Cain	zebra	Amboseli	Khan & Cain (1979)
Sporormiella similis Khan & Cain	rabbit, cow, zebra, antelope	Mt Kenya NP, L. Amboseli, Amboseli	Khan & Cain (1979)
Sporormiella subtilis Ahmed & Cain	eland	Mt Kenya NP	Khan & Cain (1979)
Sporormiella tenuispora Khan & Cain	cow	L. Amboseli	Khan & Cain (1979)
Sporormiella teretispora Ahmed & Cain	rock hyrax	Mt Kenya NP	Khan & Cain (1979)
Sporormiella tetramera Ahmed & Cain	giraffe, cow, buffalo	Nairobi NP, L. Amboseli	Khan & Cain (1979)
Stachybotrys atra Corda var. *microspora* Mathur & Sankhla	eland	Marula Estates, Naivasha	Caretta et al.(1998)
Stilbum coprophilum Matsushima	impala	Marula Estates, Naivasha	Caretta et al.(1998)
Thecotheus africanus R.S. Khan & J.C. Krug	elephant	Shimba Hills NR	Krug & Khan (1987)
Triangularia karachiensis (S.I. Ahmed & Asad) Udagawa	buffalo	L. Amboseli	Khan & Krug (1989b)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Trichobolus sphaerospora Kimbrough	antelope	Ngong Road Forest	Minoura (1969)
Trichosporiella sporotrichoides van Oorschot	bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Tripterospora erostrata (Griffiths) Cain	elephant	L. Amboseli	Khan & Krug (1989b)
Acremoniella verrucosa Tognini	reedbuck, steenbok, zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Acremonium sp.	steenbok	Marula Estates, Naivasha	Caretta et al.(1998)
Acremonium strictum W. Gams	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Actinomucor elegans (Eidam) Benjamin & Hesseltine	reedbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Alternaria alternata (Fr.) Keissler	reedbuck, bushbuck, waterbuck, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Arthrobotrys oligospora Fres.	reedbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Ascobolus americanus (Cke. et Ellis) Seaver	antelope	Ngong Road Forest	Minoura (1969)
Ascobolus immersus Pers.	reedbuck, steenbok, impala, waterbuck, buffalo, zebra, dikdik	Marula Estates, Naivasha	Caretta et al.(1998)
Ascobolus perplexans Massee & Salmon	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Ascobolus sp.	dikdik	Marula Estates, Naivasha	Caretta et al.(1998)
Ascobolus viridulis Phill. et Plow.	cow	Ngong Road Forest	Minoura (1969)
Ascodesmis nigricans van Tieghen	antelope	Ngong Road Forest	Minoura (1969)
Ascophanus lacteus (Cke. et Phill.) Sacc.	carnivore	Ngong Road Forest	Minoura (1969)
Bipolaris cynodontis (Marignoni) Shoemaker	impala, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Camarosporium sp.	reedbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Chaetomium bostrychodes Zopf	reedbuck, steenbok, impala, bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Chaetomium convolutum Chivers	bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Chaetomium crispatum (Fuckel)	soil,wheat straw	Nairobi KARI labs	Gatumbi & Kungu (1994)
Chaetomium fusiformes Chivers	antelope	Ngong Road Forest	Minoura (1969)
Chaetomium globosum Kunze ex Fr.	antelope	Ngong Road Forest	Minoura (1969)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Chaetomium mareoticum (Waheeb & Hasan)	soil, wheat straw	Nairobi KARI labs	Gatumbi & Kungu (1994)
Chaetomium murorum Corda	soil, wheat straw, waterbuck, zebu	Nairobi KARI labs Marula Estates, Naivasha	Gatumbi & Kungu (1994) Caretta et al. (1998)
Chaetomium olivaceum (Cooke & Ellis)	soil, wheat straw	Nairobi, KARI labs	Gatumbi & Kungu (1994)
Chaetomium subspirales Chivers	antelope	Ngong Road Forest	Minoura 1969
Chaetomium undulatum Bainer	soil, wheat straw	Nairobi, KARI labs	Gatumbi & Kungu (1994)
Cladorrhinum foecundissimum Sacc. & Marchal	impala, waterbuck, dikdik	Marula Estates, Naivasha	Caretta et al. (1998)
Cladosporium cladosporioides (Fresen.) de Vries	bushbuck, waterbuck, hippo, buffalo	Marula Estates, Naivasha	Caretta et al.(1998)
Cladosporium sp.	bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Coprinus sp.	steenbok, impala, bushbuck, zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Coprotus disculus Kimbr. Luck-Allen & Cain	impala	Marula Estates, Naivasha	Caretta et al.(1998)
Coprotus niveus (Fuckel) Kimbr.	impala, waterbuck, hippo, buffalo, dikdik,	Marula Estates, Naivasha	Caretta et al.(1998)
Curvularia lunata (Wakker) Boedjn	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Dactylaria sp.	bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Doratomyces columnaris Swart	zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Doratomyces stemonitis (Pers.:Fr.) Morton & G. Smith	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Endophragmiella dimorphospora (Awao & Udagawa) S.J. Hughes	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Epicoccum purpurascens Ehrenb	hippopotamus, buffalo	Marula Estates, Naivasha	Caretta et al.(1998)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Fusarium chlamydosporium Wollenw. & Reinking	reedbuck, impala, bushbuck, hippopotamus, zebra	Marula Estates, Naivasha	Caretta et al.(1998)
Fusarium sp.	reedbuck, steenbok, waterbuck, zebra	Marula Estates, Naivasha	Caretta et al.(1998)
Gelasinospora brevispora R.S. Khan & J.C. Krug	cow, zebra	Voi, Shimba Hills, Amboseli, L. Amboseli	Khan & Krug (1989a)
Gelasinospora dictyophora R.S. Khan & J.C. Krug	antelope, cow	Aberdares Ranges, Kajiado	Khan & Krug (1989a)
Gelasinospora hapsidophora R.S. Khan & J.C. Krug	elephant	Mt Kenya NP	Khan & Krug (1989a)
Gliocladium roseum Bainier	reedbuck, steenbok, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Iodophanus cameus (Pers.) Korf	steenbok, impala, bushbuck, waterbuck, buffalo, zebra, dikdik, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Kernia nitida (Sacc.) Nieuwland	reedbuck, waterbuck, dikdik, zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Lasiobolus lasioboloides Marchal	steenbok, impala, bushbuck,waterbuck, hippopotamus, zebra, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Melanospora zamiae Corda	steenbok, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Metarhizium anisopliae (Metschn.) Sorok	steenbok, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Microsphaeropsis olivacea (Bonord.) Hohn.	hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Mucor hiemalis Wehmer	zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Mucor sp.	buffalo	Marula Estates, Naivasha	Caretta et al.(1998)
Myrothecium roridum Tode ex Steudel	bushbuck, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Myrothecium verrucaria (Albertini & Schwein.) Ditm. ex Steudel	steenbok	Marula Estates, Naivasha	Caretta et al.(1998)
Oedocephalum glomerulosum (Buill.) Sacc.	reedbuck, steenbok, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Phialophora sp. (anamorph of *Podospora conica*)	bushbuck, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Pilobolus crystallinus (Wiggers) Tode	reedbuck, steenbok, impala, bushbuck, waterbuck, hippopotamus, buffalo, zebu, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Pilobolus sp.	impala, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Podosordaria phoenicia	zebra	N/K	Krug & Cain (1974)
Podospora aloides (Fuckel) Mirza & Cain	buffalo	Mt Kenya NP	Krug & Khan (1989)
Podospora anserina (Ces. ex Rab.) Niessl.	zebra, cow, reedbuck, steenbok,	Nairobi NP, L. Amboseli	Krug & Khan (1989)
[*Podospora pauciseta* (Ces.) Traverso]	impala, bushbuck, waterbuck, hippopotamus, buffalo, zebra, dikdik, zebu, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora argentinensis (Speg.) Mirza & Cain	cow	Mt Kenya NP	Krug & Khan (1989)
	zebra	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora australis (Speg.) Niessl.	zebra, giraffe, cow, steenbok, impala, bushbuck hippopotamus, buffalo,	Nairobi NP, Kajiado, L. Amboseli	Krug & Khan (1989)
	zebra, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora austro-americana (Speg.) Mirza & Cain	rock hyrax, carnivore	Mt Kenya NP, Machakos	Krug & Khan (1989)
Podospora caligata R.S. Khan & Cain	antelope, cow	Nairobi NP, Mt Kenya NP	Krug & Khan (1989)
	cow	Kajiado	Khan & Cain (1972)
Podospora comata Milovtzova	reedbuck, steenbok, hippopotamus	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora communis (Speg.) Niessl.	zebra, buffalo, wildebeest, cow, elephant	Aberdare Ranges, Nairobi NP, Mt Kenya NP, Kajiado, Voi, Amboseli	Krug & Khan (1989)
Podospora conica (Fuckel) Bell & Mahoney	impala, bushbuck, hippopotamus, eland	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora curvicolla (Winter) Niessl	duiker	n/k	Lundqvist (1973)
Podospora curvispora (Cain) Cain	elephant, cow, rock hyrax	Mt Kenya NP	Krug & Khan (1989)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Podospora curvuloides Cain	cow	Mt Kenya NP	Krug & Khan (1989)
	n/k	n/k	Lundqvist (1973)
Podospora decipiens (Winter) Niessl	buffalo, cow	Aberdares Ranges, Mt Kenya NP, Kajiado	Krug & Khan (1989)
Podospora deropodalis R.S. Khan & Cain	cow, zebra	Kajiado, Mombasa,	Krug & Khan (1989)
	zebra	Amboseli, L. Amboseli	Khan & Cain (1972)
Podospora fimicola Ces.	buffalo, eland	Mt Kenya NP	Krug & Khan (1989)
Podospora fimiseda Ces.	antelope	L .Amboseli	Krug & Khan (1989)
Podospora hyalopilosa (Stratton) Cain	antelope, cow, zebra, buffalo	Nairobi NP, Kajiado, Machakos, Mtito Andei, Amboseli, L. Amboseli	Krug & Khan (1989)
Podospora longicaudata (Griff.) Cain	cow, antelope, buffalo	Kajiado, Voi, Mombasa, L. Amboseli	Krug & Khan (1989)
Podospora millespora (A. Schimdt) Cain	antelope	Ngong Road Forest	Minoura (1969)
Podospora multispora R.S. Khan & Cain	wildebeest	Nairobi NP	Krug & Khan (1989)
	n/k	n/k	Khan & Cain (1972)
	cow	Kajiado	Khan & Cain (1972)
Podospora ostilingospora Cain	cow	Machakos	Krug & Khan (1989)
Podospora papillata Khan & Cain	n/k, zebra, elephant	N/K, L. Amboseli	Khan & Cain (1972)
Podospora perplexans (Cain) Cain	buffalo	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora platensis (Speg.) Niessl.	cow, elephant	Voi	Krug & Khan (1989)
Podospora pleiospora (Winter) Niessl.	elephant, buffalo	Aberdare, Mt Kenya NPs	Krug & Khan (1989)
Podospora prethopodalis Cain	zebra, unknown herbivore, antelope, elephant, rabbit	Nairobi NP, Amboseli, L. Amboseli	Krug & Khan (1989)
	n/k	n/k	Lundqvist (1973, 1981)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Podospora setosa (Winter) Niessl.	bushbuck, zebra, giraffe, antelope, cow steenbok, bushbuck	Aberdare Ranges, Nairobi NP, Mt Kenya NP	Krug & Khan (1989)
	antelope	Marula Estates, Naivasha	Caretta et al.(1998)
		Ngong Road Forest	Minoura (1969)
Podospora similis (Hansen) Niessl	zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Podospora spinulosa Khan & Cain	giraffe, cow, zebra, buffalo cow, elephant	Nairobi NP, Mt Kenya NP, Mtito Andei,	Krug & Khan (1989)
		Mombasa, L. Amboseli, L. Amboseli	Khan & Cain (1972)
Podospora tetraspora (Winter) Cain	rock hyrax	Mt Kenya NP	Krug & Khan (1989)
Ramichloridium apiculatum (Miller, Giddens & Foster) de Hoog	hippopotamus, buffalo, zebu	Marula Estates, Naivasha	Caretta et al.(1998)
Saccobolus depauperatus (Berk. et Br.) Phill.	antelope	Ngong Road Forest	Minoura (1969)
Saccobolus versicolor (P. Karst.) P. Karst.	reedbuck, steenbok, waterbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Saccobolus violascens Boud.	antelope	Ngong Road Forest	Minoura (1969)
Sepedonium niveum Massee & Salmon	steenbok	Marula Estates, Naivasha	Caretta et al.(1998)
Sordaria alcina Lundq.	zebra	Aberdares Ranges	Khan & Krug (1989b)
Sordaria brevicollis L.S. Olive & Fantini	zebra, buffalo	Mtito Andei, L. Amboseli	Khan & Krug (1989b)
Sordaria fimicola (Rob.) Ces. & De Not.	antelope, elephant, buffalo, eland, rock hyrax, cow, zebra steenbok	Aberdare Ranges, Aberdare N.P., Nairobi NP, Mt Kenya NP, Machakos, Mtito Andei, Mombasa, Voi, Amboseli, L. Amboseli	Khan & Krug (1989b)
		Marula Estates, Naivasha	Caretta et al.(1998)
Sordaria humana (Fuckel) Winter	buffalo	L. Amboseli	Khan & Krug (1989b)
	goat and antelope	Ngong Road Forest	Minoura (1969)

Table 2.1 (Continues)

Fungal species	Dung type/Substrate	Location	Reference
Sordaria lappae Poteb.	antelope, wildebeest, elephant, buffalo, cow, eland, rock hyrax, zebra	Aberdare N.P., Aberdare Ranges, Nairobi NP, Mt Kenya NP, Amboseli, L. Amboseli	Khan & Krug (1989b)
Sordaria superba De Not.	bushbuck, wildebeest, elephant	Aberdare Ranges, Nairobi NP, Mt Kenya NP	Khan & Krug (1989b)
Sordaria tomento-alba Cailleux	antelope, bushbuck, elephant, zebra	Aberdare Ranges, Mt Kenya NP, Mtito Andei, Amboseli	Khan & Krug (1989b)
Sporomiella minima (Auersw.) Ahmed & Cain	reedbuck, steenbok, impala, bushbuck, waterbuck, hippopotamus, buffalo, zebra, dikdik, zebu, eland	Marula Estates, Naivasha	Caretta *et al*.(1998)
Sporormiella australis (Speg.) Ahmed & Cain	rock hyrax, eland, elephant, antelope, zebra, cow	Aberdare Ranges, Nairobi NP, Mt Kenya NP	Khan & Cain (1979)
Sporormiella capybarae (Speg.) Ahmed & Cain	cow, buffalo	L. Amboseli	Khan & Cain (1979)
Sporormiella dubia Ahmed & Cain	giraffe, buffalo, zebra, cow	Nairobi NP, Mt Kenya NP, Amboseli, L. Amboseli	Khan & Cain (1979)
Sporormia fimetaria De Not.	cow, antelope, zebra, buffalo	Kajiado, Voi, Mombasa, Amboseli, L. Amboseli	Khan & Cain (1979)
Sporormia fimicola S.I. Ahmed & Asad	cow	L. Amboseli	Khan & Cain (1979)
Sporormiella herculea (Ell. & Ev.) Ahmed & Cain	cow	L. Amboseli	Khan & Cain (1979)
Sporormiella intermedia (Auersw.) Ahmed & Cain	antelope, rock hyrax, zebra, buffalo	Nairobi NP, Mt Kenya NP, Amboseli	Khan & Cain (1979)
Sporormiella isomera Ahmed et Cain	antelope, elephant, carnivore, zebra antelope	Nairobi NP, Mt Kenya NP, Mtito Andei, L. Amboseli, Ngong Road Forest	Khan & Cain (1979) Minoura (1969)
Sporormiella kansensis (Griff.) Ahmed & Cain	antelope	L. Amboseli	Khan & Cain (1979)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Sporormiella longispora (Cain) Ahmed & Cain	giraffe	Nairobi NP	Khan & Cain (1979)
Sporormiella longisporosis Ahmed & Cain	zebra	Amboseli	Khan & Cain (1979)
Sporormiella macropulchella Khan & Cain	cow	L. Amboseli, Mt Kenya NP	Khan & Cain (1979)
Sporormiella megalospora (Auersw.) Ahmed & Cain	rock hyrax	Mt Kenya NP	Khan & Cain (1979)
Sporormiella minima (Auersw.) Ahmed & Cain	buffalo, duiker, antelope, elephant, zebra, cow, rock hyrax	Aberdare Ranges, Nairobi &, Mt Kenya NPs, Mtito Andei, Kajiado, Voi, Machakos, Mombasa, L. Amboseli	Khan & Cain (1979)
Sporormiella minimoides Ahmed & Cain	unidentified herbivore	Nairobi NP	Khan & Cain (1979)
Sporormiella muskokensis Ahmed & Cain	rock hyrax	Mt Kenya NP	Khan & Cain (1979)
Sporormiella obliqua Khan & Cain	cow, antelope, wildebeest, rabbit	L. Amboseli, Mt Kenya NP	Khan & Cain (1979)
Sporormiella pilosa (Cain) Ahmed & Cain	zebra	Amboseli	Khan & Cain (1979)
Sporormiella similis Khan & Cain	rabbit, cow, zebra, antelope	Mt Kenya NP, L. Amboseli, Amboseli	Khan & Cain (1979)
Sporormiella subtilis Ahmed & Cain	eland	Mt Kenya NP	Khan & Cain (1979)
Sporormiella tenuispora Khan & Cain	cow	L. Amboseli	Khan & Cain (1979)
Sporormiella teretispora Ahmed & Cain	rock hyrax	Mt Kenya NP	Khan & Cain (1979)
Sporormiella tetramera Ahmed & Cain	giraffe, cow, buffalo	Nairobi NP, L. Amboseli	Khan & Cain (1979)
Stachybotrys atra Corda var. *microspora* Mathur & Sankhla	eland	Marula Estates, Naivasha	Caretta et al.(1998)
Stilbum coprophilum Matsushima	impala	Marula Estates, Naivasha	Caretta et al.(1998)
Thecotheus africanus R.S. Khan & J.C. Krug	elephant	Shimba Hills NR	Krug & Khan (1987)
Triangularia karachiensis (S.I. Ahmed & Asad) Udagawa	buffalo	L. Amboseli	Khan & Krug (1989b)

Table 2.1 (continues)

Fungal species	Dung type/Substrate	Location	Reference
Trichobolus sphaerospora Kimbrough	antelope	Ngong Road Forest	Minoura (1969)
Trichosporiella sporotrichoides van Oorschot	bushbuck	Marula Estates, Naivasha	Caretta et al.(1998)
Tripterospora erostrata (Griffiths) Cain	elephant	L. Amboseli	Khan & Krug (1989b)

Coprophilous ascomycetes is ubiquitous and forms a very important component of biodiversity in conservation areas. The coprophilous of dung fungi is closely related to the ecology of animals that support them (Lundqvist, 1972; Bell, 1983, 2005). Richardson (2004) points out that coprophilous fungi species diversity is lower in harsh and degraded environments such as Kenya's semi-arid north-eastern region. To ensure as much ascomycetes diversity as possible was observed in this study, sampling sites were selected in different ecosystems, namely, highland grasslands, coastal rain forests, highland rain forests and lowland grasslands.

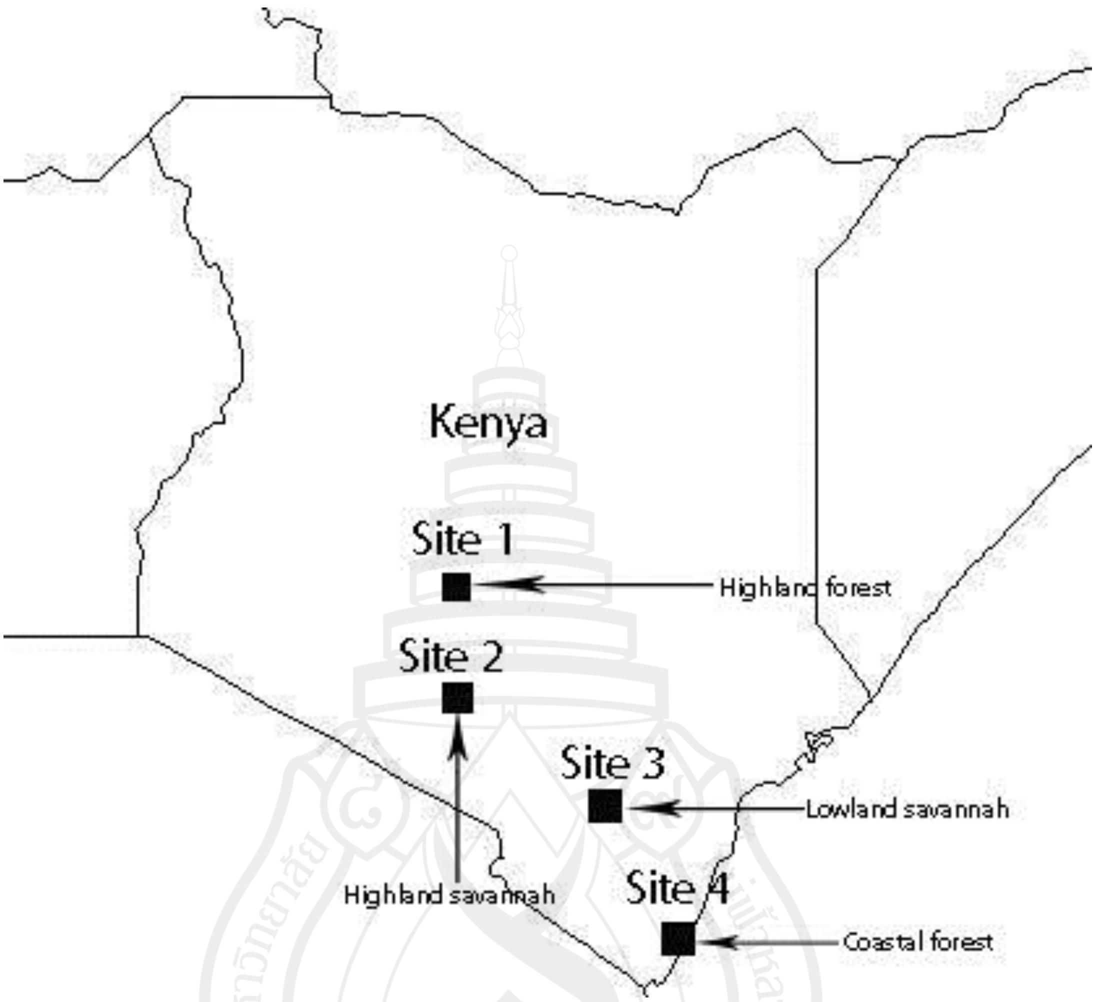
Our dung samples had either fine or coarse structure and were voided as pile(s) or pellets. Coprophilous ascomycetes were cultured by a moist chamber method. The field and laboratory work for this study was undertaken between April 2008 and October 2011. Classification of the ten genera of coprophilous ascomycetes isolated was mainly based on the morphology of the specimens. Dichotomous keys and monographs were extensively used to classify the coprophilous ascomycetes.

2.2 Materials and Methods

2.2.1 Study Sites

Conservation areas in Kenya represent diverse ecosystems that harbour varying fauna and flora species. Site selection was based on habitat type, herbivore species diversity and the climate of the site. The distance between collection sites was an important parameter taken into consideration to ensure that there was minimal overlap of dung from migratory animal species.

Each wild animal species has a characteristic dung structure. Some groups of coprophilous fungi show a preference for growth on particular types of dung therefore it was necessary to sample as many different wild herbivore species from diverse habitats (Lundqvist, 1972; Bell, 1983). Sampling sites were thus selected in Nairobi N.P., Shimba Hills N.R., Aberdares N.P. and Tsavo N.P. (Figure 2.1 and Table 2.2).



Note. (Site 1) Aberdare National Park and adjacent Country Club Game Sanctuary. (Site 2) Nairobi N.P. (Site 3) Tsavo East N.P. (Site 4) Shimba Hills and Kinondo Forest Reserves.

Figure 2.1 Map of Kenya Showing Wildlife Dung Sampling Sites.

Table 2.2 Description of Dung Sampling Sites

Sampling site	GPS	Habitat type and conditions	Common wildlife species
Aberdares N.P. and Aberdares Country Club Game Sanctuary (size 767 km^2)	Between E36°31’ and E36°57’ and between S0°08 and S0°42’ Altitude 1920-4001 m above sea level	Tropical highland forest Rainfall: bimodal 900-2000 mm p.a. Mean maximum temp. 18^{0}C Mean minimum temp. 6^{0}C	Elephant, waterbuck, giant forest hog, giraffe, black rhinoceros, zebra, impala, eland, duiker, warthog, suni, sykes monkey, reedbuck, dikdik, bongo
Nairobi N.P. (size 117 km^2)	S2°18’ to S2°20’ and E36°23’ to E36°28’ Altitude 1600-790 m above sea level	Highland savannah Rainfall: bimodal 762-911 mm p.a. Mean maximum temp. 24.8 to 25.4^{0}C - Mean minimum temp. - 12.3 to 13.1^{0}C	Cape buffalo, giraffe, zebra, impala, hippopotamus, waterbuck, Jackson’s hartebeest, black rhinoceros, white rhinoceros, lion, wildebeest, eland, leopard, hippopotamus, cheetah, Grant’s gazelles, Thomson’s gazelles, baboons, Coke’s hartebeest
Tsavo East N.P. (size 4,150 km^2)	E38°30’ to E39°15’ and S2°45’ Altitude 200-550 m above sea level	Lowland savannah Rainfall: bimodal, 110-700 mm p.a. Mean maximum temp. 27-33^{0}C Mean minimum temp. 17-21^{0}C	Elephant, Cape buffalo, impala, lion, giraffe, waterbuck, eland, cheetah, suni, elephant, gerenuk, squirrel, zebra, oryx, porcupine, leopard, black rhinoceros, ant bear, tree hyrax, rock hyrax, lesser kudu, warthog, steenbok, Coke’s hartebeest, red duiker, blue duiker, bush duiker, klipspringer, kirk’s dikdik, reedbuck
Shimba Hills (size 192.51 km^2) and Kinondo Forest Reserves (size 0.3 km^2)	Shimba Hills E39°17’ to E39°30’ and S4°09’ to S4°21’ Altitude 105 to 448 m above sea level Kinondo Forest 4°23'41"S 39°32'44"E Altitude 0 to 10 m above sea level	Coastal tropical forests Rainfall: bimodal, Mean rainfall 1151 mm p.a. Mean maximum temp. 20.4^{0}C Mean minimum temp. 28.2^{0}C	Dikdik, Cape buffalo, giraffe, impala, sable antelope, warthog, bushbuck, Jackson’s hartebesst, bushpig, hyena, elephant, Rothschild jackal, giraffe, yellow baboon, reedbuck,leopard, colobus monkey, waterbuck

2.2.2 Dung Sample Collection

Identifying the animal voiding dung was an important aspect of this study. Wild animals whose dung was sampled were common species and were identified directly when encountered or by indirect means such as signs, tracks and the physical appearance and the size of dung pile or pellet (Figure 2.2). The local knowledge of animals by Kenya Wildlife Service game rangers was useful in the identification of any unknown wild animals. Dung samples were collected from the African elephant (*Loxodonta Africana* Blumenbach), Cape buffalo (*Syncerus caffer* Sparrman), giraffe (*Giraffa camelopardalis* Linnaeus), zebra (*Equus burchellii* Boddaert), hippopotamus (*Hippopotamus amphibius* Linnaeus), Kirk's dikdik (*Madoqua kirki* Günther), waterbuck (*Kobus ellipsiprymnus* Ogilbyi), impala (*Aepyceros melampus* Lichtenstein), giant forest hog (Hylochoerus meinertzhageni Thomas), sable antelope (*Hippotragus niger* Harris), Jackson's hartebeest (*Alcelaphus buselaphus* Pallas), bushbuck (*Tragelaphus scriptus* Pallas) and black rhinoceros (*Diceros bicornis* Linnaeus). The dung samples collected were either fine or coarse structured and were voided as either pile(s) or pellets.

Field dung sampling was done in April 2008, April 2009, August 2010 and October 2011. Sampling site variables comprising geographic coordinates of substrate location were taken using a Garmin® GPS or a Hewlett Packard (HP®) GPS enabled Personal Digital Assistant (PDA). Moisture condition of dung, animal species voiding dung, vegetation type, soil, weather details, collection date and the name of the collector were recorded in a datasheet. Wet dung samples were air-dried at site to ensure there was no sporulation during transportation from the field. For each dung type sampled, about 50-100gm of dung material was collected, placed in a clean sterile container or envelope labeled with a unique number and returned to the KWS laboratory in Nairobi.

Note. (A) Fresh hippopotamus dung. (B) Fresh elephant. (C) Dikdik dung. (D) Giraffe dung. (E) Dikdik dung. (F) Fresh buffalo dung. (G) Fresh waterbuck dung. (H) Dry zebra dung. (I) Fresh giraffe dung. (J) Heap of fresh and old dikdik dung.

Figure 2.2 Dung Types and Their Relative Size.

Fifty-five dung samples were collected randomly from various conservation areas and 13 different wild animal species (Table 2.3). Out of these, four dung samples were collected from Aberdare Country Club Game Sanctuary, four from Aberdares National Park, one from Kinondo Forest Reserve, 25 from Nairobi National Park, six from Shimba Hills National Reserve and 15 from Tsavo East National Park. In regard to animal species, rhinoceros and elephant had four dung samples each; nine samples from buffalo; ten from giraffe; six each for impala and zebra; hippopotamus and dikdik had three each; five were from waterbuck; bushbuck, giant forest hog and sable antelope had one each; hartebeest had two. Each sampling site had animal species that were generally representative of that particular habitat type.

Table 2.3 Distribution of Dung Types and Samples by Study Sites

Site	Elephant	Buffalo	Giraffe	Zebra	Hippo	Dikdik	Water buck	Impala	Giant forest hog	Sable antelope	Hartebeest	Bushbuck	Rhino	Total
Aberdare Country Club Game Sanctuary			1	1				1					1	**4**
Aberdare National Park	1						1		1				1	**4**
Kinondo Forest Reserve						1								**1**
Nairobi National Park		4	6	5	3		1	3			1		2	**25**
Shimba Hills National Reserve		1	1					1		1	1	1		**6**
Tsavo East National Park	3	4	2			2	3	1						**15**
Total	**4**	**9**	**10**	**6**	**3**	**3**	**5**	**6**	**1**	**1**	**2**	**1**	**4**	**55**

2.2.3 Coprophilous Ascomycetes Culture, Isolation and Examination

Culturing was done at the Kenya Wildlife Service laboratory by moist chamber technique. The moist chambers were made of petri dishes fitted inside the base with filter paper or paper towel which served to maintain moistness throughout the incubation period. Prior to incubation all the dung samples were examined for signs of sporulation (Bell, 1983, 2005; Doveri, 2004). Each dung sample was divided into two portions, one for herbarium preservation and the other for laboratory culture.

Each portion for incubation was put in its own moist chamber, moistened with sterile water, ensuring that it did not become too wet. The moist chambers were placed on window sills on the side of the laboratory facing sunrise and kept under normal room temperature and natural light to induce sporulation of coprophilous ascomycetes (Brummelen van, 1967; Lundqvist, 1972; Bell, 1983, 2005; Richardson, 2002; Doveri, 2004; Krug, 2004).

After about five days of incubation, the surface of the dung was examined with a dissecting microscope for fruiting bodies (Bell, 1983, 2005). This was done on a regular basis to monitor each of the successive ascomycetes sporulating. Fruiting bodies seen were picked off individually with a clean and sterile fine tipped forceps or probe and placed in a drop of sterile water on a clean microscope slide, gently squashed with the rubber tip of a pencil to spread out fungal structures for observation under high magnification (Doveri, 2004). Standard stains such as Melzer's, Indian ink, Congo red and lactophenol blue were applied to slide mounts to help examine important diagnostic fungal structures (Bell, 1983, 2005).

The plates were moistened regularly to ensure continued fungal sporulation (Lundqvist, 1972; Bell, 2005). Each batch of culture was maintained and examined for approximately three months when it became apparent that no further significant ascomycetes sporulation would occur.

2.2.4 Characterization of Coprophilous Ascomycetes

To classify coprophilous ascomycetes digital images and photomicrographs of the fungal characters such as ascomata, ascospores, paraphyses, ascomatal wall were taken with stereo and compound microscopes equiped with cameras and operated through computer software. The size of fungal structures, such as, ascospores, asci, ascomata, paraphyses and peridial cells were measured using the image framework© software. Adobe Photoshop© software was used to process and analyze the images. Permanent slides were made in lactic acid and sealed with clear nail varnish (Bell, 2005).

Each species was examined in detail and important variables such as sample number, dung type, date of incubation, date of examination, tentative name, notes on reaction with stains, habit and colour, photograph number, the age of dung when the mature ascomata was picked recorded in a laboratory data sheet.

Dichotomous keys were used to circumscribe species from isolates examined (Cain, 1934; Brummelen van, 1967; Mirza & Cain, 1969; Lundqvist, 1972; Ahmed & Cain, 1972; Korf, 1972; Khan & Cain, 1979; Bell, 1983, 2005; Richardson & Watling, 1997; Hansen et al., 1998; Doveri, 2004, 2008; Chang & Wang, 2005). The identification was further confirmed by making a comparison with specimens described in referenced work (Prokhorov, 1991; Wang, 1993, 1997, Doveri, 2004).

2.2.5 Data Analysis

Herbarium material was prepared by placing small pieces of air-dried dung in paper envelopes and cryotubes (Bell, 1983, 2005; Doveri, 2004). Each sample was labeled carefully and put in a paper envelope and placed in a dry cabinet for preservation. A virtual herbarium, consisting of all digital images taken in the course of this study, was developed. These are preserved at the KWS headquarters database and laboratory for future reference.

To compute the occurrence of the isolated taxa, the percentage and relative frequency of each taxon was computed using the formulae below:

$$\text{Frequency (\%)} = \frac{\text{Sample units on which fungal species occurred}}{\text{Total number of sample units examined}} \times 100$$

$$\text{Relative frequency (\%)} = \frac{\text{Number of isolates for each species}}{\text{Total number of isolates}} \times 100$$

2.3 Results and Discussion

2.3.1 Diversity of Coprophilous Ascomycetes

Table 2.4 shows the distribution and diversity of coprophilous ascomycetes by collection site and also animal producing the dung. A total of 127 ascomycetes specimens belonging to ten genera were isolated. These genera comprised twenty-eight species, among them, six of *Ascobolus*, one of *Arnium*, five of *Podospora*, seven of *Saccobolus*, five of *Schizothecium*, five of *Chaetomium*, eight of *Sporormiella*, one of *Sordaria*, two of *Zopfiella* and one of *Zygopleurage* (Table 2.5).

The most common coprophilous ascomycetes from wildlife dung were *Podospora communis* with a frequency of 16.4% and a relative frequency of 14.4%, *Saccobolus depauperatus* a frequency of 14.5% and relative frequency of 10.3%, *Zygopleurage zygospora* with a frequency of 10.9% and relative frequency of 10.3%, *Ascobolus calesco* with a frequency of 12.7% and relative frequency of 7.2%, *Podospora anserina* with a frequency of 9.1% and relative frequency of 7.2%) while *Ascobolus amoenus* had a frequency of 9.1% and relative frequency of 6.2%. *Ascobolus bistisii*, *Schizothecium curvuloides* var. *curvuloides*, *S. dakotense*, *Podospora australis* and *Saccobolus citrinus* were also fairly common in wildlife dung.

The genus *Arnium* and *Sordaria* each with a single species and isolate seem to occur rarely in wildlife dung. Within *Saccobolus*, *S. infestans* was observed only once. *Ascobolus* had a tendency to sporulate only on fresh dung that had not been subjected to preservation. *Saccobolus*, *Schizothecium* and *Podospora* were observed on both fresh and preserved dung. Giraffe, buffalo, zebra, dikdik and impala dung were observed to be preferred substrates for a majority of coprophilous ascomycetes examined. An interesting finding was that from just three dikdik dung samples a very diverse range of ascomycetes was isolated.

Table 2.4 Distribution of Ascomycetes Examined by Sampling Site and Dung Type

National Park or Reserve	Dung types	Ascomycetes species
Aberdares NP and Aberdares Country Club Game Sanctuary	Elephant, waterbuck, giant forest hog, giraffe, black rhinoceros, zebra, impala	*Ascobolus calesco, A. amoenus, Sacobolus depauperatus, S. diffusus, S. platensis, Podospora anserina, P. argentinensis, P. australis, P. communis, P. minor, Schizothecium curvuloides* var. *curvuloides, S. dakotense, Zygopleurage zygospora, Sporormiella intermedia, Sporormiella* aff. *minipascua, Sporormiella* aff. *muskokensis, Sporormiella* aff. *teretispora, Chaetomium muelleri, C. seminis-citrulli, Chaetomium* sp.
Nairobi NP	Buffalo, giraffe, zebra, impala, hippopotamus, waterbuck, hartebeest, black rhinoceros	*Ascobolus calesco, A. bistisii, A. amoenus, A. immersus, A. nairobiensis, Saccobolus citrinus, S. depauperatus, S. infestans, S. truncatus, S. versicolor, Podospora australis, P. anserina, P. argentinensis, P. communis, Schizothecium conicum, S. curvuloides* var. *curvuloides, S. dakotense, S. dubium, Arnium arizonense, Zygopleurage zygospora, Sporormiella herculea, S. intermedia, S. longisporopsis, S. minima, Sporormiella* aff. *minipascua, Chaetomium convolutum, C. globosum*
Tsavo East NP	Elephant, buffalo, impala giraffe, dikdik, waterbuck	*Ascobolus amoenus, A. calesco, A. tsavoensis, Saccobolus depauperatus, S. platensis, S. truncatus, Podospora communis, P. anserina, Zopfiella* aff. *erostrata, Zopfiella longicaudata, Zygopleurage zygospora, Sporormiella minima, Chaetomium convolutum*
Shimba Hills and Kinondo Forest Reserves	Dikdik, buffalo, giraffe, impala, sable antelope, hartebesst, bushbuck	*Saccobolus depauperatus, Schizothecium glutinans, Zygopleurage zygospora, Sordaria fimicola, Sporormiella leporina, S. minima, Sporormiella* sp.

Table 2.5 Distribution of Ascomycetes Taxa Examined by Dung Type

Dung type	Coprophilous ascomycetes species
Elephant	*Ascobolus amoenus* (11 and 12 days*), *A. calesco* (6 and 8 days), *Saccobolus depauperatus* (14, 44, 45 days), *S. platensis* (18, 59 days), *Zopfiella longicaudata* (80 days), *Zygopleurage zygospora* (40, 79 days), *Podospora anserina* (6, 20), *P. communis* (12-64 days)
Waterbuck	*Ascobolus amoenus* (11 and 12 days), *A. tsavoensis* (11 days), *Saccobolus diffusus* (72 days), *S. platensis* (18, 59 days), *Zygopleurage zygospora* (40, 79 days), *Schizothecium dakotense* (12, 39, 40, 50), *Podospora communis* (12-64 days).
Cape buffalo	*Ascobolus amoenus* (11 and 12 days), *A. calesco* (6 and 8 days), *Saccobolus citrinus* (18, 19 days), *S. depauperatus* (14, 44, 45 days), *Zygopleurage zygospora* (40, 79 days), *Schizothecium conicum* (19 days), *Podospora anserina* (6, 20), *P. communis* (12-64 days)
Impala	*Ascobolus amoenus* (11 and 12 days), *A. bistisii* (10, 12 days), *A. immersus* (10, 20 days), *Saccobolus citrinus* (18, 19 days), *S. depauperatus* (14, 44, 45 days), *S. truncatus*, 54, 57 days), *S. versicolor* (18 days), *Zygopleurage zygospora* (40, 79 days), *Schizothecium curvuloides* var. *curvuloides* (41,42,50, 54 days) *S. dakotense* (12, 39, 40, 50), *S. dubium* (80 days), *Podospora communis* (12-64 days)
Zebra	*Ascobolus amoenus* (11 and 12 days), *A. bistisii* (10, 12 days), *A. calesco* (6 and 8 days), *S. depauperatus* (14, 44, 45 days), *S. infestans* (59 days), *Zygopleurage zygospora* (40, 79 days), *Schizothecium curvuloides* var. *curvuloides* (41,42,50, 54 days), *S. dakotense* (12, 39, 40, 50), *Podospora argentinensis* (34, 43 days), *P. australis* (12, 21 days), *Podospora communis* (12-64 days), *P. minor* (42 days)
Giraffe	*Ascobolus bistisii* (10, 12 days), *A. calesco* (6 and 8 days), *A. immersus* (10, 20 days), *Saccobolus citrinus* (18, 19 days), *S. depauperatus* (14, 44, 45 days), *S. truncatus*, 54, 57 days), *Arnium arizonense* (33 days), *Schizothecium dakotense* (12, 39, 40, 50), *Podospora communis* (12-64 days)
Black rhinoceros	*Ascobolus calesco* (6 and 8 days), *A. immersus* (10, 20 days), *A. nairobiensis* (10 days), *Podospora anserina* (6, 20), *Podospora communis* (12-64 days).

Table 2.5 (continues)

Dung type	Coprophilous ascomycetes species
Hippopotamus	*Ascobolus calesco* (6 and 8 days), *Saccobolus citrinus* (18, 19 days), *Schizothecium curvuloides* var. *curvuloides* (41, 42,50, 54 days), *Podospora anserina* (6, 20), *Podospora communis* (12-64 days).
Dikdik	*Ascobolus calesco* (6 and 8 days), *Saccobolus depauperatus* (14, 44, 45 days), *Zopfiella* aff. *erostrata* (14), *Zopfiella longicaudata* (80 days), *Sordaria fimicola* (14), *Podospora anserina* (6, 20).
Bushbuck	*Saccobolus depauperatus* (14, 44, 45 days).
Hartebeest	*Saccobolus depauperatus* (14, 44, 45 days), *Zygopleurage zygospora*(40, 79 days), *Schizothecium curvuloides* var. *curvuloides* (41, 42,50, 54 days)
Sable antelope	*Schizothecium glutinans* (54 days).
Giant forest hog	*Podospora communis* (12-64 days).

Note. *Number in brackets denotes incubation period in days

2.3.2 Coprophilous Ascomycetes Succession

The sporulation time was divided into 11 weekly periods starting with 0-7 days to 71-77 days (Table 2.6). Ascomycetes that sporulated were fitted into the relevant weekly period (cohort). The pattern observed was that sporulation started with *Ascobolus* followed by *Saccobolus, Schizothecium, Podospora, Arnium, Sordaria, and Zopfiella* and ended with *Zygopleurage*.

Podospora communis, *Saccobolus depauperatus* and *Iodophanus difformis* seem to sporulate across the entire incubation period. Apart from within *Ascobolus* it was observed that there was sequential sporulation amongst the other genera.

Age of incubated dung was observed and it was noted that some species of the genera *Saccobolus* namely *S. diffusus, S. infestans, S. truncatus* and *S. versicolor* mainly sporulated at 40 to 70 days, which was the last period of incubation. *Saccobolus depauperatus,* the most common species was observed to sporulate across the entire period. *Sporormiella minima* and *S. intermedia* did not seem to follow any particular pattern or period. *Zopfiella* aff. *erostrata* sporulated in the first period while *Zopfiella longicaudata* sporulated in the last period of incubation and only on dung from Tsavo East National Park, a rather dry savannah.

Table 2.6 Succession in Wildlife Dung Ascomycetes

Taxa	Isolation time (days)										
	0-7	8-14	15-21	22-28	29-35	36-42	43-49	50-56	57-63	64-70	71-77
Arnium arizonense		1			1						
Ascobolus albidus		1									
Ascobolus amoenus	1	5									
Ascobolus bistisii		5									
Ascobolus calesco	4	3	1								
Ascobolus immersus		2									
Cercophora sp											2
Chaetomium globosum	1	3									
Iodophanus difformis		1	1							2	
Lasiobolus intermedius	1	2									
Podospora anserina	4	4	2								
Podospora argentinensis					1		1				
Podospora australis		1									
Podospora communis			2			4					1
Podospora minor		1									
Saccobolus citrinus			3		1						
Saccobolus depauperatus		1					3				
Saccobolus versicolor			1								
Saccobolus diffusus											1
Saccobolus infestans									2		
Saccobolus platensis			1						1		

Table 2.6 (continues)

Taxa	**Isolation time (days)**										
	0-7	**8-14**	**15-21**	**22-28**	**29-35**	**36-42**	**43-49**	**50-56**	**57-63**	**64-70**	**71-77**
Saccobolus truncatus								1	1		
Schizothecium curvuloides						1		1			
Schizothecium dakotense		3									
Schizothecium dubium											1
Sordaria fimicola		2									
Sporormiella intermedia						1			4		
Sporormiella minima		7			1						
Zopfiella longicaudata										2	1
Zopfiella aff. erostrata	1	1	1								
Zygopleurage zygospora						1	1			2	2

Note. Time taken is the age of the dung from incubation date to mature fruit body isolation

2.4 Conclusion

Moist chamber cultures of wildlife dung yielded 127 ascomycetes specimens belonging to ten genera. These specimens were isolated and characterized using the morphological technique. The genera comprised twenty-eight species, among them, six of *Ascobolus*, one of *Arnium*, five of *Podospora*, seven of *Saccobolus*, five of *Schizothecium*, five of *Chaetomium*, eight of *Sporormiella*, one of *Sordaria*, two of *Zopfiella* and one of *Zygopleurage.*

The occurrence, distribution and diversity of coprophilous ascomycetes from wildlife dung appear to be influenced by dung and habitat types. Some species such as those from the genus *Ascobolus* only sporulate on fresh dung incubated immediately after collection. The structure of dung also had an influence on what type of ascomycetes sporulated.

Irrespective of feeding habits and browse height, dung from all animals was able to support the growth of coprophilous ascomycetes. The phenomenon of succession was observed within the various taxa of coprophilous ascomycetes. It was noted that various species sporulated on dung in a definite pattern thus showing the classical gradual appearance and disappearance of fruit bodies, hyphae and mycelia on the surface of the substrate.

Apparently, wildlife dung is a very rich substrate as far as coprophilous fungi are concerned. More diverse dung types will definitely yield more taxa of ascomycetes. Dung from browsing animals appeared to harbour more ascomycetes fungi diversity. More research into this is required so as to determine the validity of this observation.

The dung sampled and incubated came in various structures and composition, for instance Cape buffalo and giant forest hog dung is voided as fine piles, while elephant and hippopotamus void are very coarse large piles. Zebra voids fairly large pellets while giraffe, waterbuck, sable antelope, hartebeest, impala, bushbuck and dikdik void smaller pellets in that order.

Some rarely studied dung types such as the dikdik, giant forest hog, Jackson's hartebeest and sable antelope were examined. Species such as *Saccobolus infestans*, *Arnium arizonense*, *and Sordaria fimicola* appeared quite rarely on wildlife dung.

Dung from giraffe, a high canopy browser, has quite a remarkable diversity of coprophilous ascomycetes. Some genera of coprophilous ascomycetes displayed a preference for certain dung types, for instance, *Chaetomium* and some *Sprormiella* clearly showed a preference for browsing animal dung.

CHAPTER 3

COPROPHILOUS ASCOMYCETES: I. *Ascobolus* Pers.

3.1 Introduction

Ascobolus comprising mostly cosmopolitan and coprophilous species that sporulate on dung within the first week of incubation is made up of over 60 species (Bell, 2005; Kirk et al., 2008). The pale-yellow luteous ascomata are superficial, gregarious and sessile but sometimes can be with a short stalk of up to 30 mm diameter (Brummelen van, 1967; Doveri, 2004; Bell, 2005). Paraphyses are often filiform, cylindrical and usually embedded in mucus. Asci are usually 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 usually 4 or 8-spored (Doveri, 2004; Bell, 2005). 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).

Ascobolus has been recorded in North America, South America, Europe, the Middle East, Australia, New Zealand, Asia and Russia (Brummelen van, 1967; Liou & Chen, 1977; Bell, 1983, 2005; Prokhorov, 1991; Abdullah & Alutbi, 1993; Wang, 2000; Mireille et al., 2002; Doveri, 2004; Richardson, 2008). In Africa, *Ascobolus albidus* P. Crouan & H. Crouan, *A. amoenus* Oudem., *A. degluptus* Brumm., *A. hawaiiensis* Brumm., *A. immersus* Pers. and *A. stictoideus* Speg. have been recorded in Uganda, South Africa and Egypt (Minoura, 1969; Ebersohn & Eicker, 1992, 1997; Abdel-Azeem et al., 2005; Abdel-Azeem, 2009). Kenyan records of species of this genus include *Ascobolus americanus* (Cooke & Ellis) Seaver, *A. immersus* Pers., *A.*

perplexans Massee & Salmon, *A. viridulus* W. Phillips & Plowr. and an undetermined *Ascobolus* sp. (Minoura, 1969; Caretta et al., 1998).

This survey sought to identify and classify *Ascobolus* species found on various dung types in Kenyan wildlife and to develop a checklist for species associated with different wildlife dung types.

3.2 Materials and Methods

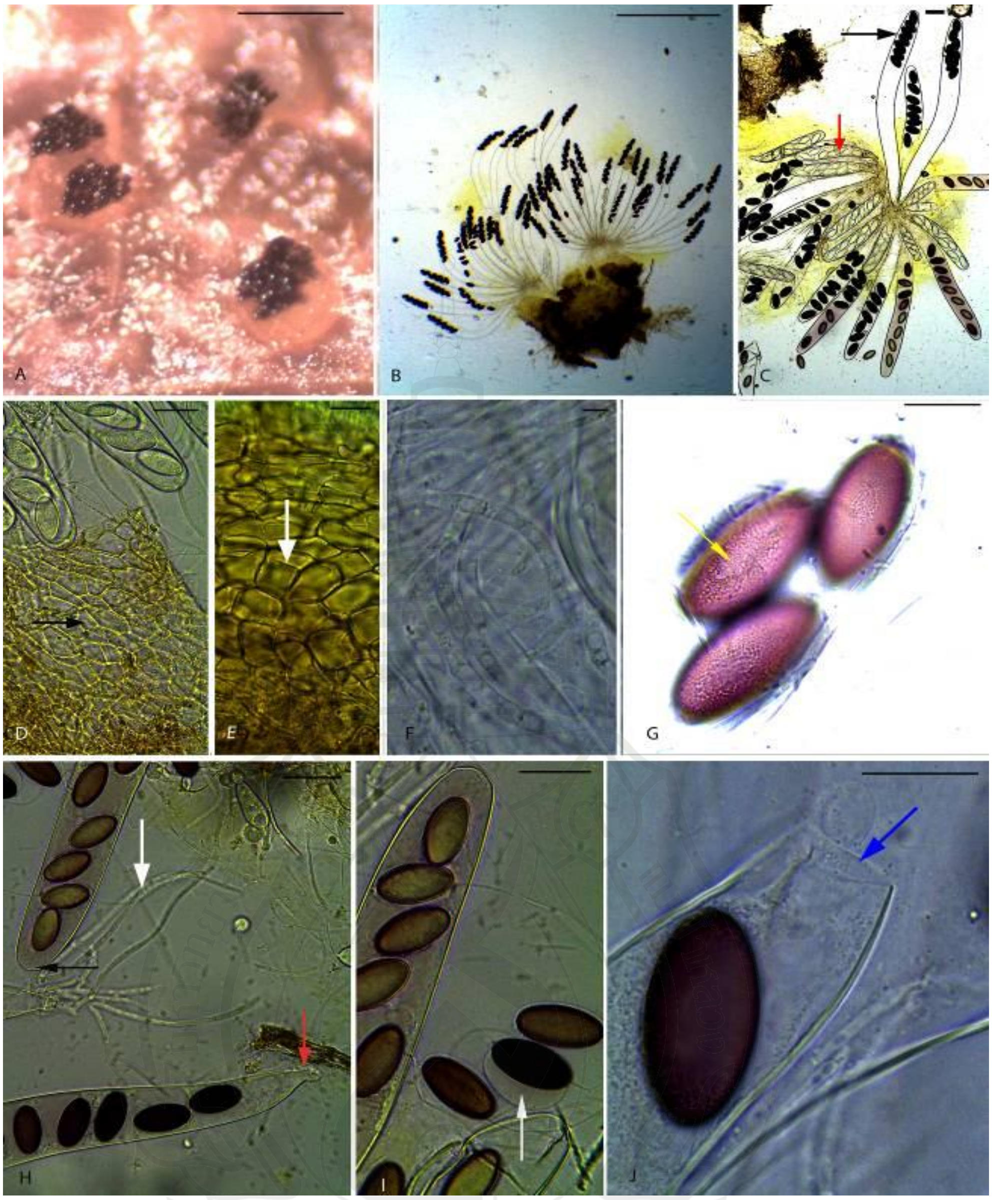
Wildlife dung samples were incubated and screened for *Ascobolus* species using the procedures described in Section 2.2 of Chapter 2. The *Ascobolus* species isolated were classified based on morphological characters as described in monographs and dichotomous keys on members of the family Ascobolaceae (Brummelen van, 1969; Korf, 1972; Bell, 1983, 2005; Richardson & Watling, 1997; Hansen et al., 1998; Doveri, 2004).

3.3 Results and Discussion

Ascobolus belongs to the family Ascobolaceae Boud. ex Sacc. comprising species usually having apothecioid ascomata, amyloid 8-spored asci, asci that protrude way beyond the hymenium at maturity, 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 & Schumacher, 1994). Each individual ascopore is usually enveloped by a mucilaginous sheath and/or strongly ornamented (Brummelen van, 1967; Bell, 2005). *Ascobolus* species are often saprobic and coprophilous (Bell, 2005). The sections below give details of each species examined in this study.

3.3.1 *Ascobolus amoenus* Oudem., Hedwigia 21: 165 (1882). (Figure 3.1A-J)

Ascomata at first cleistothecioid, 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.



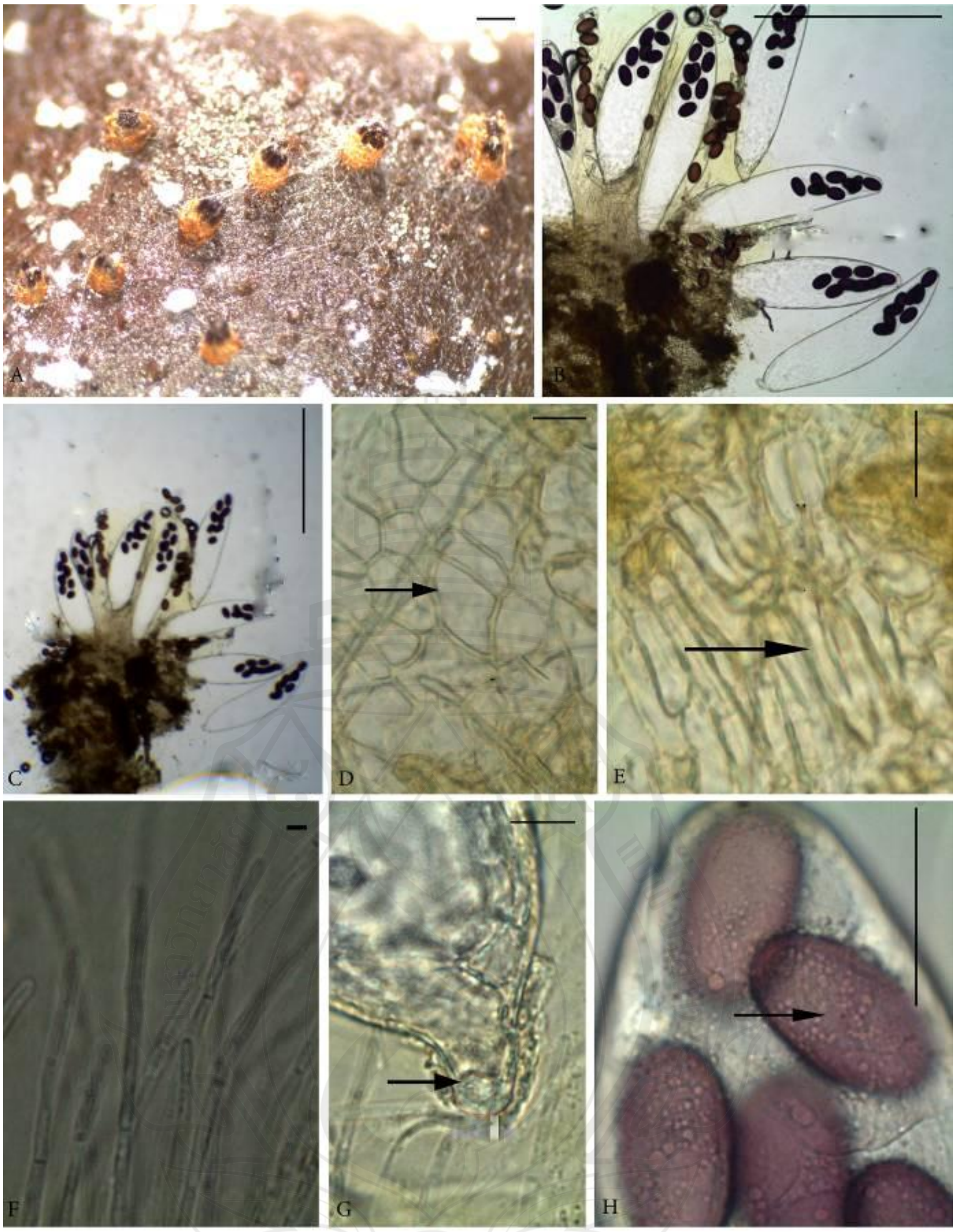
Note. (A) Ascomata on substrate. (B) Ascoma squashed in water. (C) Detail of hymenium with 8-spored mature asci (black arrow) and immature asci (red arrow). (D-E) Details of ectal excipulum. Note irregular shape (black arrow) and isodiametric cells (white arrow). (F) Paraphyses. (G) Dotted-granular ascospores. (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 µm, (C) = 200 µm, (D, H) = 50 µm, (E-G, J) = 20 µm.

Figure 3.1 *Ascobolus amoenus* (KWSTE003B-2009).

The characters of *Ascobolus amoenus* Sect. *Dasyobolus* (Sacc.) Brumm. agree with those provided for the same species by Brummelen van (1967), Minoura (1969), Jahn (1997) and Bell (2005). *Ascobolus amoenus* is differentiated from *A. elegans* (J. Klein) Brumm. by its relatively smaller ascospores. This species has previously been recorded in Kenya as *Ascobolus americanus* (Minoura, 1969).

3.3.2 *Ascobolus bistisii* Gamundí & Ranalli, Nova Hedwigia 10: 347 (1966). (Figure 3.2A-H)

Ascomata cleistothecioid in the early stages with a hymenium exposed only late, superficial to semi-immersed, sessile, 200–500 µm diameter. *Receptacle* globular, dotted with few protruding asci, light brown at first, finally brown, glabrous, becoming barrel shaped, margin hardly differentiated. *Disc* convex, light greenish yellow to brown. *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, septate, 2.5–4 µm broad, 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, short stipitate, 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.



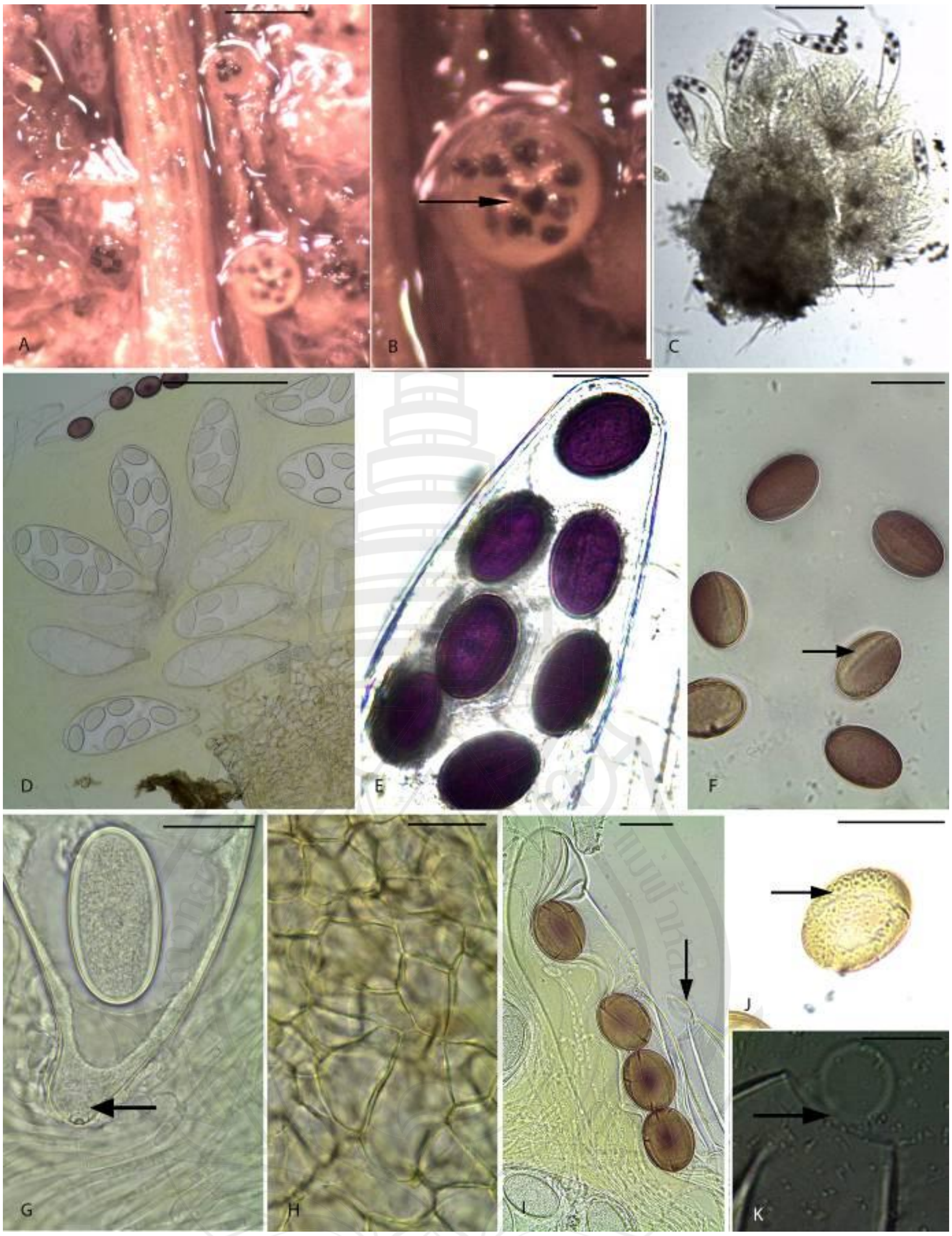
Note. (A) Ascomata on dung. (B) Mature asci and ascospores. (C) Squashed ascoma. (D-E) Details of ectal excipulum, note shape of cells and elongated cells near the margin (arrows). (F) Paraphyses. (G) Ascus stipe, (arrow). H Free mature ascospores, note oil droplets (arrow). Scale bars: (A) = 1000 µm,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

Figure 3.2 *Ascobolus bistisii* (KWSNP020-2010).

Ascobolus bistisii Sect. *Dasyobolus* is a fairly common species on Kenyan wildlife dung. This species is morphologically similar to *Ascobolus immersus*. However, the smaller sized spores, type of ornamentation coupled with the unilateral gelatinous sheath prove that our specimen is *Ascobolus bistisii* (Richardson & Watling, 1997; Mireille *et al*. 2002; Doveri, 2004; Bell, 2005). Our specimen was a new record for Kenya.

3.3.3 ***Ascobolus calesco*** A.E. Bell & Mahoney, Fungal Planet, no. 21: 22, (2007). (Figure 3.3A-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, vacuoled, 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.



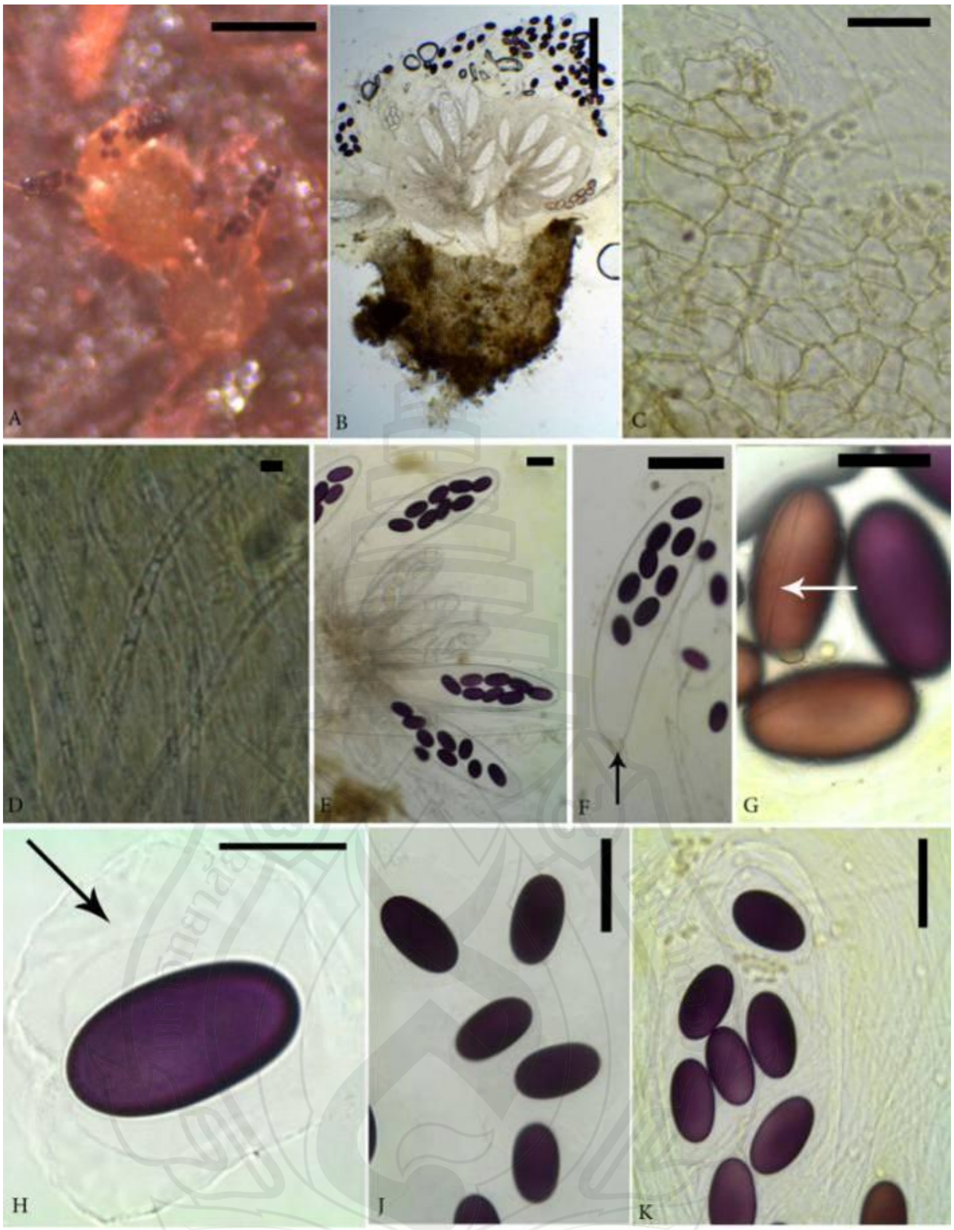
Note. (A-B) Ascomata on substrate, note black asci tips (arrow). (C) Ascoma squashed mount. (D) Free asci with spores. (E) Ascus tip with spores. (F) Free ascospores, note ornamentation (arrow). (G) Paraphyses and ascus stipe (arrow). (H) Details of ascomatal wall. (I) Ascus apex showing an open operculum (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, I-K) = 50 µm, (G-H) = 20 µm.

Figure 3.3 *Ascobolus calesco* (KWSTE005B-2009).

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 that has a description very similar to this Kenyan collection is mentioned. Bell & Mahoney (2007) clarified the correct taxonomic position with a new species examined from Australia which she named *Ascobolus calesco*. Our Kenyan collection has ascospores within the size range 43–50 × 31–36 µm which compares well with *Ascobolus calesco* A.E. Bell & Mahoney. This was a new record for Kenya.

3.3.4 *Ascobolus immersus* Pers., Neues Mag. Bot. 1:115 (1794). (Figure 4A-K)

Ascomata cleistothecioid 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 uncinate, 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.



Note. (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 µm, (C, J-K) = 50 µm, (D, G-H) = 20 µm, (E-F) = 200 µm.

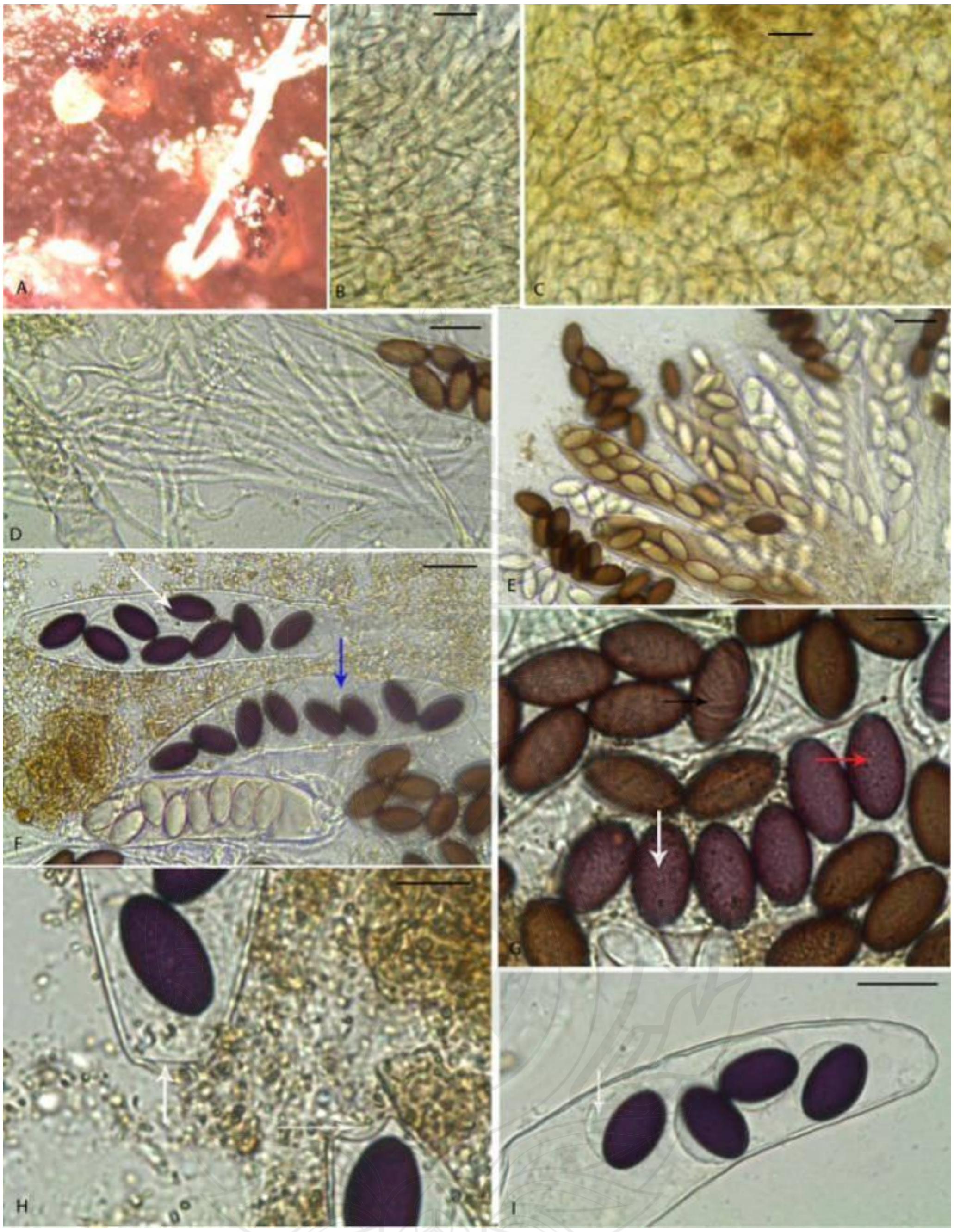
Figure 3.4 *Ascobolus immersus* (KWSNNP020-2010).

Ascobolus immersus Sect. *Dasyobolus* is very similar to *A. bistisii* Gamundi & Ranali (Brummelen van, 1967; Bell, 2005). The two taxa are however differentiated by *A. bistisii* having ascospores that are smaller and finely warted (Brummelen van, 1967; Mireille et al., 2002; Bell, 2005) while those of *A. immersus* are larger, adorn striations and are more rounded at the ends (Brummelen van, 1967; Richardson & Watling 1997; Hansen et al., 2001; Doveri, 2004; Bell, 2005). *Ascobolus immersus* is also easily differentiated from other *Ascobolus* of Sect. *Dasyobolus* (cleistothecioid ascomata, opening at late maturity only, or not opening at all) by its comparatively larger ascomata, asci and ascospores, the latter typically smooth or with thin crevices (Brummelen van, 1967; Doveri, 2004). *Ascobolus immersus* is a very common coprophilous species on wildlife dung sporulating very early on incubation.

3.3.5 *Ascobolus nairobiensis* Mungai & K.D. Hyde, (Figure 3.5A-I) MycoBank: MB564303

Ascomata apothecioid, 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 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, 5.5–16.5 × 4–12.5 µm. *Paraphyses* filiform, simple or sometimes branched, septate, numerous, intertwined, exceeding the asci, 2.5–4 µm diam., containing hyaline vacuoles, curved above with slightly swollen tips, embedded in a colorless mucus. *Asci* 160–190 × 26–34 µm, 8-spored, cylindric-claviform, rounded above, with the wall staining blue in Melzer's reagent, operculum 9.5–13.5 µm diam., with a stipe 40–60 × 6.5–8 µm. *Ascospores* 21–26.5 × 12–14 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.

Ascobolus nairobiensis is similar to *Ascobolus albidus* and *A. furfuraceous* Pers.: Fr. However, notable differences with *Ascobolus albidus* include having shorter and broader asci and smaller ascospores. According to Brummelen van (1967) *Ascobolus albidus* has only biseriate ascospores but our collection has both biseriate and uniseriate ascospores. This taxon is different from *Ascobolus furfuraceous* because it has smooth, emarginate and whitish rather than furfuraceous and pigmented (yellow to yellowish-green) receptacles. In addition it has a well differentiated margin and paraphyses embedded in a colourless rather than in a yellowish gelatinous mucus (Doveri, 2004). *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 was thus described as a new species.

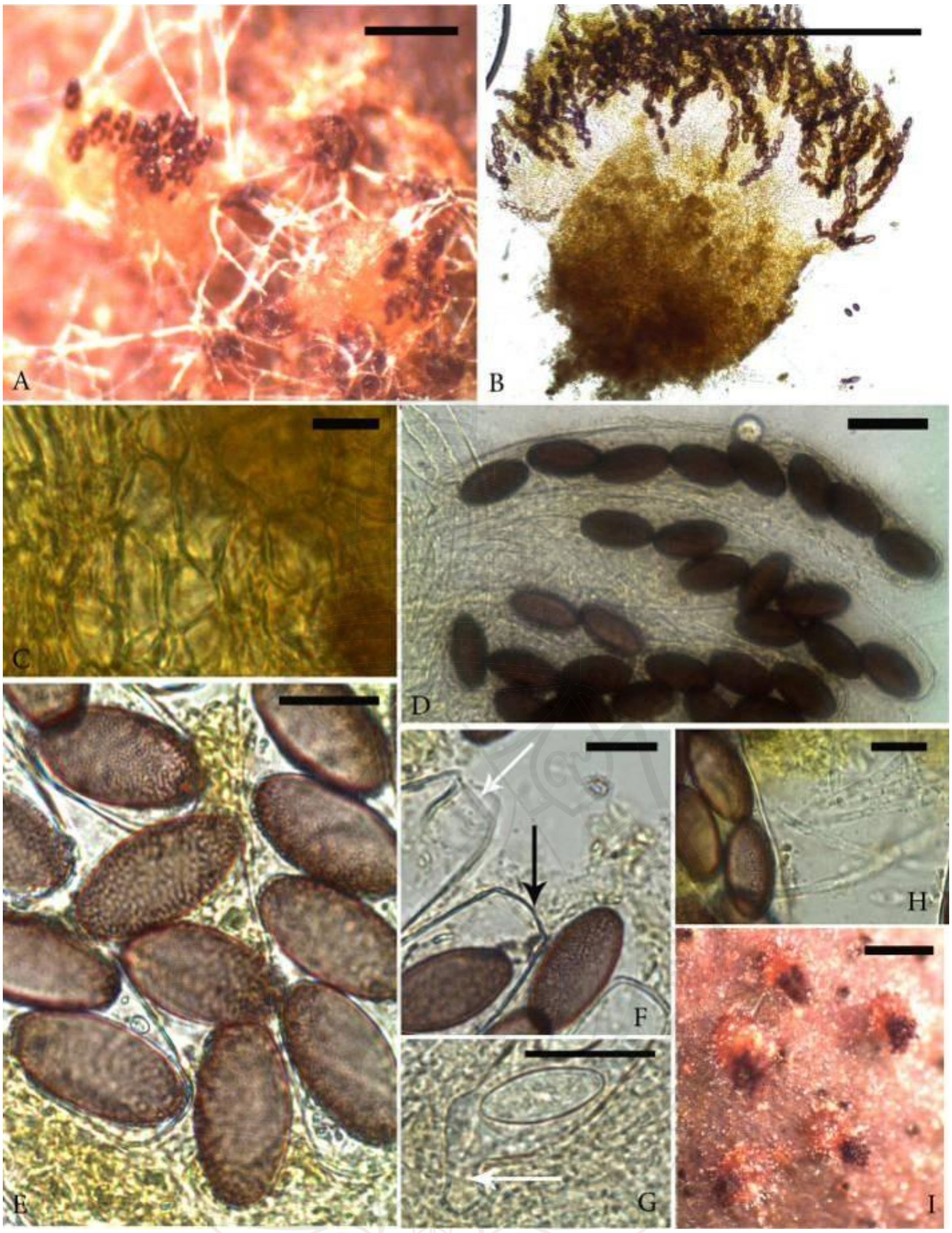


Note. (A) Ascomata on dung. (B-C) Details of ectal excipulum. (D) Paraphyses. (E-F) Asci with ascospores in different stages, note spore arrangement (white and blue arrow). (G) Free mature ascospores, note the ornamentation (arrows). (H) Apical part of asci, note the opercula (arrows). (I) Ascus apex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

Figure 3.5 *Ascobolus nairobiensis* (KWSNNP014-2010).

3.3.6 *Ascobolus tsavoensis* Mungai & K.D. Hyde, (Figure 3.6A-I) MycoBank: MB564304

Ascomata apothecioid, scattered or gregarious, superficial, sessile, 350–400 × 250–300 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 10–29.5 × 7–10 µm. *Paraphyses* filiform, 2–3 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* 200–250 × 45–55 µm, 8-spored, unitunicate, clavate-sacciform, turning deep blue in Melzer's reagent, operculate, with a dome-shaped apex, broad short stipe. *Ascospores* 26–34 × 15–18 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.



Note. (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, E-H) = 20 µm, (D) = 50 µm, (I) = 1000 µm.

Figure 3.6 *Ascobolus tsavoensis* (KWSTE006B-2009).

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 *Ascobolus bistisii*. Owing to these differences, *Ascobolus tsavoensis*, though having many features in common with *A. stictoideus* (Bell, 2005) is certainly not the same taxon. This collection constituted a new species.

3.4 Conclusions

From moist chamber cultured dung samples, twenty-two specimens of coprophilous *Ascobolus* species were isolated and examined. Three taxa, *Ascobolus calesco*, *A. amoenus* and *A. bistisii*, sporulated on a wide range of dung types and were the most common. Two of the examined species, *Ascobolus nairobiensis* and *Ascobolus tsavoensis* were new to science while *Ascobolus bistisii* and *A. calesco* were recorded for the first time in Kenya.

CHAPTER 4

COPROPHILOUS ASCOMYCETES: II. *Saccobolus* Boud.

4.1 Introduction

Saccobolus belonging to the subfamily Ascobolideae Gray and family Ascobolaceae Sacc. is made up of over 25 species (Kirk et. al., 2008; www.indefungorum.org/Names/Names.asp November 2012). *Saccobolus* comprises predominantly cosmopolitan and coprophilous species (Brummelen van, 1967; Doveri, 2004). The main features of this genus include apothecioid ascomata, clustered ascospores which in most cases have brown or purple-brownish episporic pigment at maturity with each cluster enclosed in a common gelatinous membrane (Brummelen van, 1967; Kaushal & Virdi, 1986; Doveri, 2004; Bell, 2005). The firmly clustered-together bundles of ascospores are usually arranged in regular patterns (Brummelen van, 1967). The apices of paraphyses usually produce a coloured pigment (Brummelen van, 1967; Doveri, 2004; Bell, 2005). The asci are phototropic, operculate and protrude above the hymenial surface on maturity.

Saccobolus species have been from Brazil, Australia, New Zealand, Taiwan and Europe (Brummelen van, 1967; Bell, 1983, 2005; Wang, 2000; Richardson, 2008). From Africa records of *Saccobolus* include, *S. beckii* Heimerl., *S. glaber* (Pers.) Lambotte, *S. minimus* Velen., *S. portoricensis* Seaver, *S. verrucisporus* Brumm., *S. citrinus* Boud. & Torrend and *S. glaber* (Ebersohn & Eicker, 1992; Abdel-Azeem et al., 2005). Kenyan records of species of this genus from past studies include *Saccobolus depauperatus* (Berk. & Broome) Rehm, *S. versicolor* (P. Karst.) P. Karst. and *S. violascens* Boud. (Minoura, 1969; Caretta et al., 1998).

The objectives of this section were to describe and classify *Saccobolus* species found on various dung types from Kenyan wildlife and to document their species diversity and distribution of in relation to different dung types.

4.2 Materials and Methods

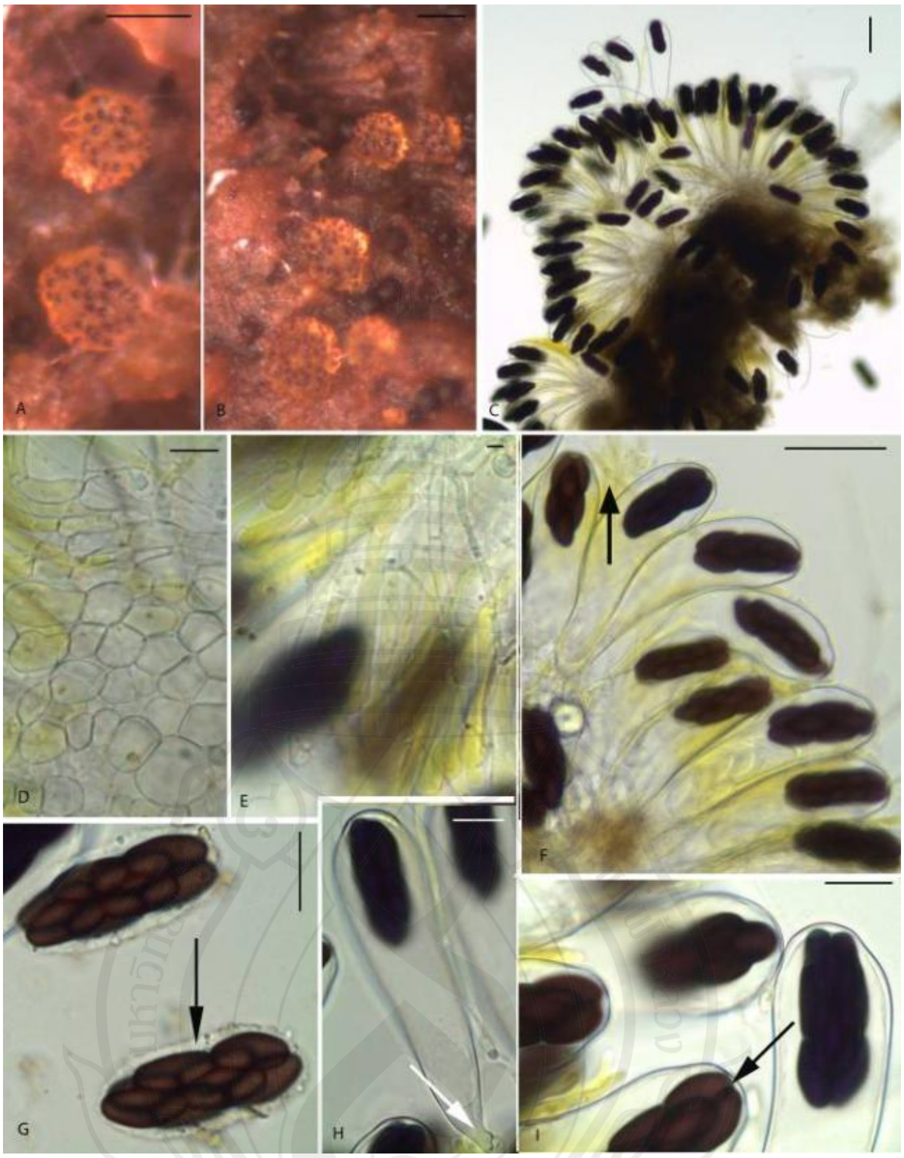
Moist chamber cultures of wildlife dung were examined for coprophilous *Saccobolus* species (see Section 2.2). *Saccobolus* species isolated were identified based on morphological characters as described in monographs and dichotomous keys on members of the family Ascobolaceae (Brummelen van, 1969; Korf, 1972; Bell, 1983, 2005; Richardson & Watling, 1997; Hansen et al., 1998; Doveri, 2004).

4.3 Results and Discussion

The main features of *Saccobolus* include apothecioid ascomata, clustered ascospores, which in most cases have brown or purple-brownish episporic pigment at maturity (Brummelen van, 1967; Kaushal & Virdi, 1986; Doveri, 2004; Bell, 2005). Each ascospore cluster is firmly enclosed in a common gelatinous membrane. The clustered bundles of ascospores are usually arranged in regular and characteristic patterns (Brummelen van, 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 former (Brummelen van, 1967; Doveri, 2004; Bell, 2005). The apices of paraphyses usually produce a coloured pigment (Brummelen van, 1967; Doveri, 2004; Bell, 2005). The asci are phototropic, operculate and protrude above the hymenial surface on maturity. Brummelen van (1967) divided *Saccobolus* into two distinct sections, namely Sect. *Saccobolus* and Sect. *Eriobolus*. Section *Saccobolus* is characterized by coloured and yellow pigment in the paraphyses while Sect. *Eriobolus* has white ascomata and colourless paraphyses. The size of structures such as ascospores and asci are important characters when delimiting species within the genus *Saccobolus*.

4.3.1 *Saccobolus citrinus* Boud. & Torrend, Bull. Soc. Mycol. Fr. 27: 131 (1911). (Figure 4.1A-I)

A*scomata* 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 made of *textura globulosa* pale yellow to yellowish-gray 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's 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.



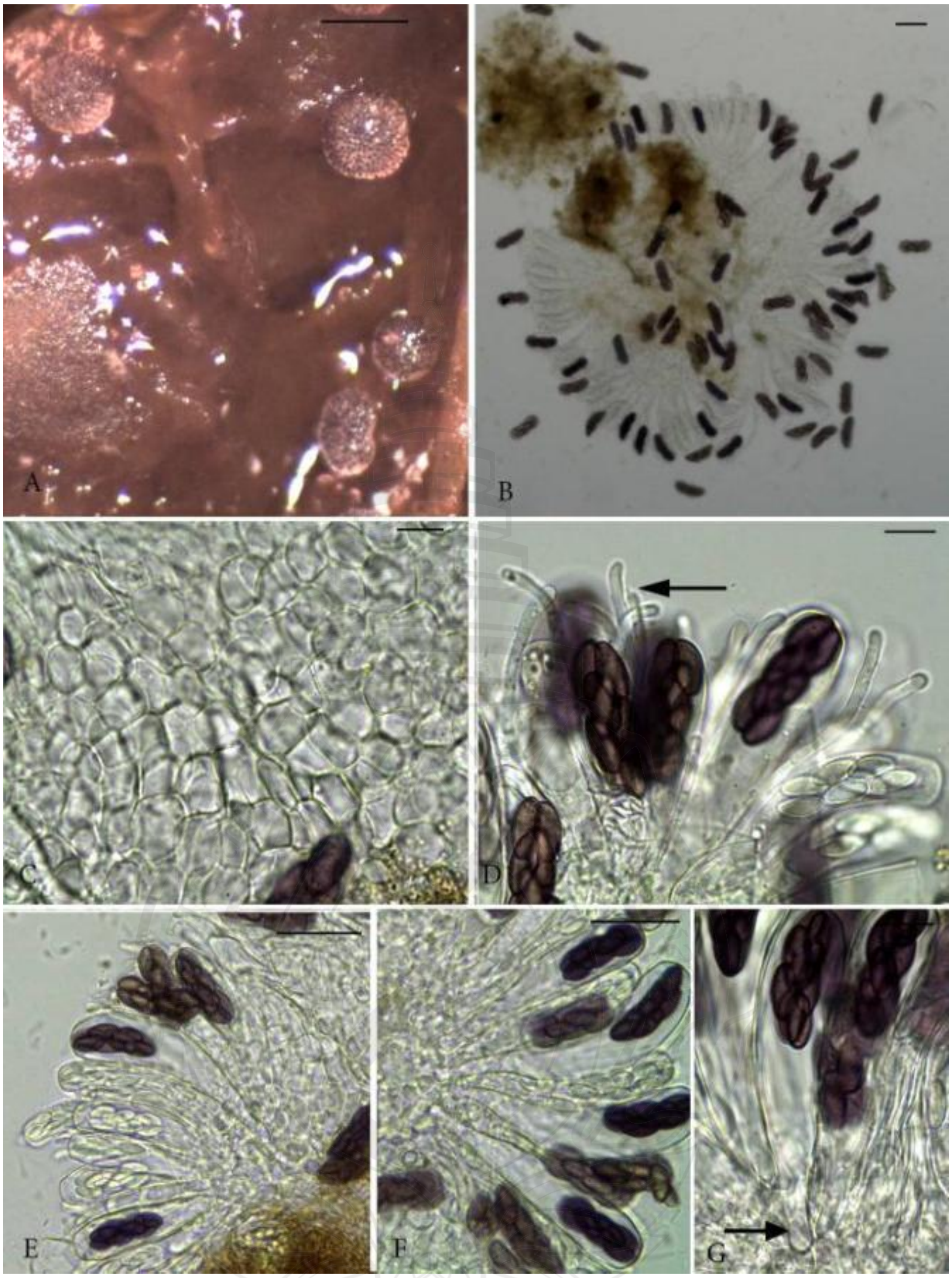
Note. (A-B) Ascomata on dung. (C) Squashed ascoma. (D) Details of ectal excipulum near the margin. (E) Paraphyses filled with yellow pigment. F Mature asci and ascospores note yellow mucus (arrow). (G) Mature ascospore clusters note gelatinous sheath (arrow). (H) Mature ascus showing a stipe (white arrow) and an apex. (I) Apical portion of asci, note closed operculum (arrow). Scale bars: (A-B) = 500 µm, (C) = 200 µm, (D, F-I) = 20 µm, (C) = 50 µm, (J) = 50 µm.

Figure 4.1 *Saccobolus citrinus* (KWSNNP020-2010).

Saccobolus citrinus Sect. *Saccobolus* is similar to *S. succineus* Brumm., (Brummelen van, 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 *Saccobolus citrinus* (21–24.5 × 8–9.5 µm in this examination) are narrower and more finely warted (Brummelen van, 1967; Doveri, 2004; Bell, 2005) while those of the latter are larger. This collection is quite a common species on wild herbivore dung in Kenya. *Saccobolus citrinus* was a new record for Kenya.

4.3.2 *Saccobolus depauperatus* Berk. & Broome., Ann. Mag. Nat. Hist. III 15: 448 (1865). (Figure 4.2A-G)

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* cylindric-filiform, septate, exceeding the asci, width 2.5–4 µm, with hyaline vacuoles; tips hyaline, not inflated. *Asci* 62–72 × 15.5–18.5 µm, 8-spored, unitunicate, claviform, often curved at apex, wall turning deep blue in Melzer's reagent, with an operculum ca.14.5 µm, long stipitate. *Ascospores* 11.5–14 × 5–7 µm, single-celled, arranged according to van Brummelen's 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 × 11.5–13.5 µm, usually surrounded by a thin hyaline gelatinous sheath.



Note. (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 µm, (B) = 200 µm, (C-D, G) = 20 µm, (E-F) = 50 µm.

Figure 4.2 *Saccobolus depauperatus* (KWSSH003-2009).

Saccobolus depauperatus Sect. *Eriobolus* is similar to *S. versicolor* (Brummelen van, 1967; Sloover de, 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 (Brummelen van, 1967; Doveri, 2004). Unlike *Saccobolus versicolor* with a *textura intricata* excipulum that of *S. depauperatus* is usually of a *textura globulosa* (Brummelen van, 1967; Sloover de, 2002; Doveri, 2004). The apices of paraphyses and excipular cells of *Saccobolus depauperatus* are also without any pigmented intercellular mucus rendering the species to appear whitish (Brummelen van, 1967; Sloover de, 2002; Doveri, 2004).

4.3.3 ***Saccobolus diffusus*** S.C. Kaushal & Virdi, Willdenowia 16 (1): 269 (1986). (Figure 4.3A-J)

Ascomata apothecioid, scattered or gregarious, superficial, sessile, 220–370 µm diam. *Receptacle* at first subglobose to globose, obconical, 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 × 4.5–8 µm. *Paraphyses* cylindric-filiform, simple or rarely branched, septate, exceeding asci, 2.5–4 µm broad; tips with abundant greenish-yellow pigmentation, not inflated. *Asci* 114–167 × 29–39 µm, 8-spored, walls turning blue in Melzer's reagent, unitunicate, broadly clavate, tapering abruptly into a short stout and lobate stipe 13–25 × 6.5–7.5 µm; apices subtruncate with an operculum 14.5–17 µm wide. *Ascospores* 21–24.5 × 8–9.5 µm, single-celled, ellipsoidal, at first hyaline, violet to brown, smooth or verruculose, 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.

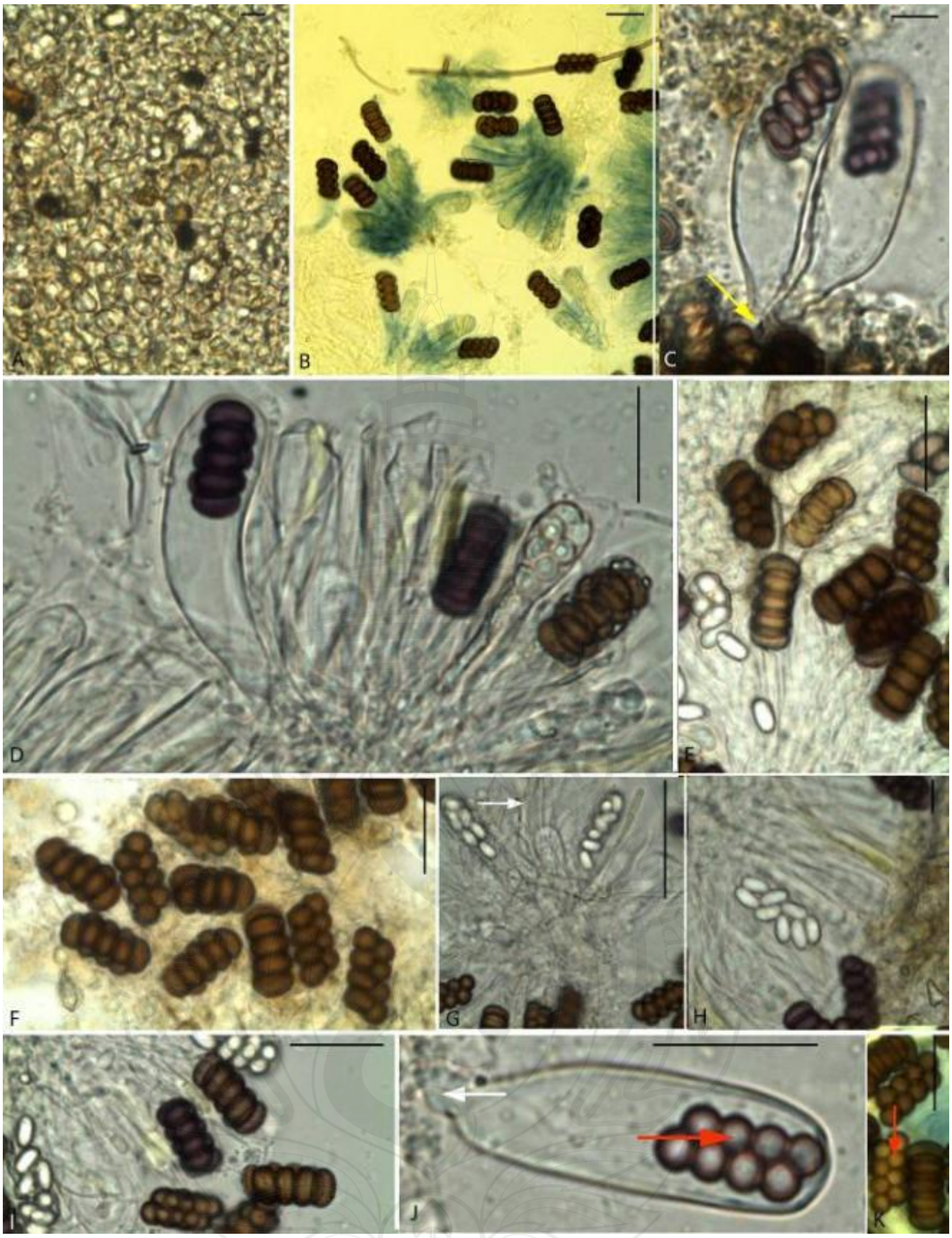
Note. (A) Ascomata on dung. (B) Squash ascoma. (C) Details of ectal excipulum. (D) Asci and ascospores among paraphyses. (C) Apical part of ascus. (F) Gelatinous envelope around ascospore cluster. (G) Free mature ascospores and a dehisced ascus. (H) Ascospore arrangement, paraphyses and hyaline immature spores in yellow mucus. (I) Ascus stipe. (J) Open operculum. Scale bars: (A) = 500 μm, (B) = 200 μm, (C, E-F, H-J) = 20 μm, (D, G) = 50 μm.

Figure 4.3 *Saccobolus diffusus* (KWSANP005-2009).

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 & Virdi, 1986; Doveri, 2004). *Saccobolus 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 & Virdi, 1986; Doveri, 2004). This was a new record for Kenya.

4.3.4 ***Saccobolus infestans*** (Bat. & Pontual) Brumm., Persoonia, Suppl. 1: 204 (1967). (Figure 4.4 A-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 bluing 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 van Brummelen's pattern VI; clusters compact, arranged in two rows, 19–25 × 9.5–11.5 µm, cemented together by a hyaline gelatinous sheath.



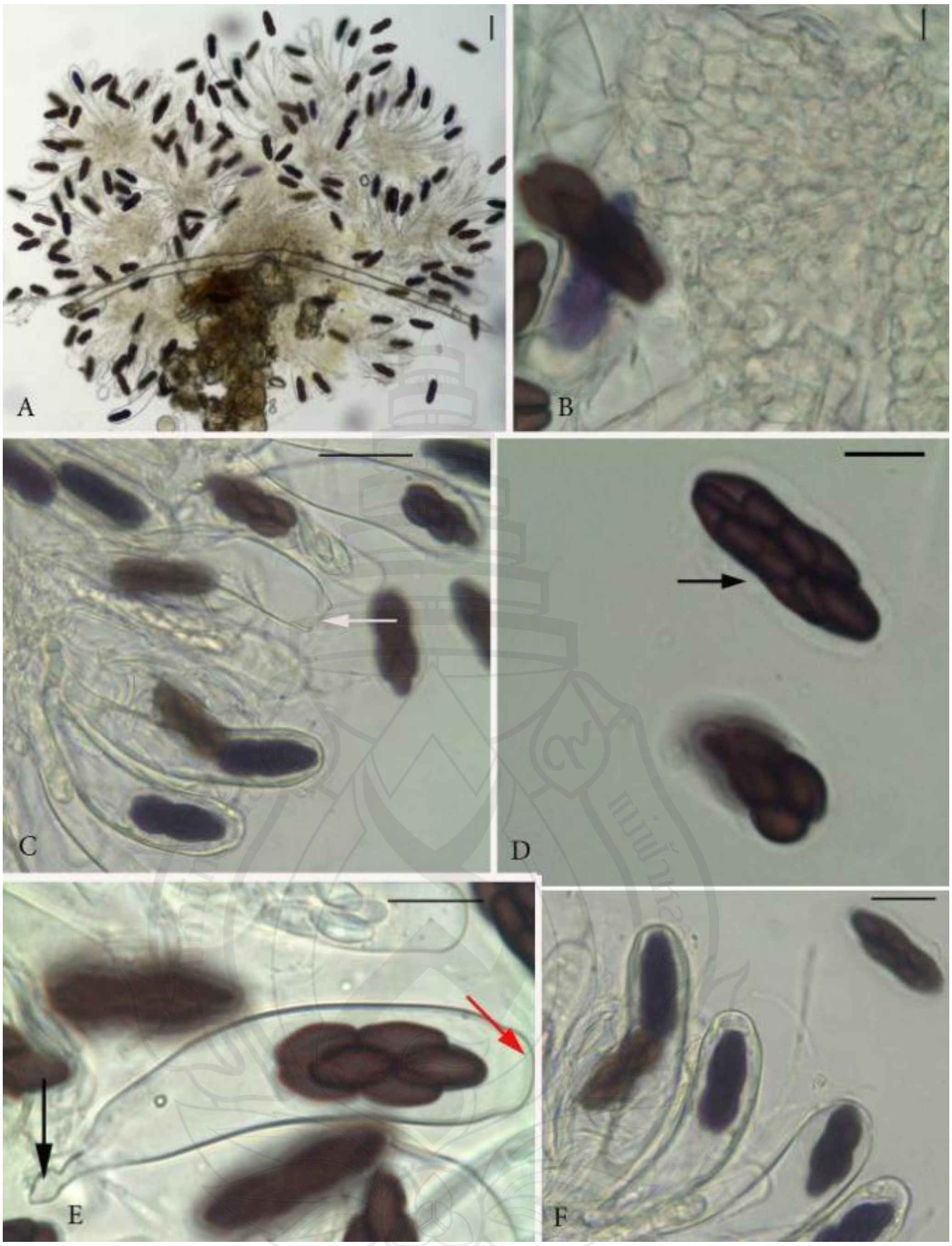
Note. (A) Details of ectal excipulum. (B) Mature asci and ascospores. (C) Mature asci showing stipe (arrow). (D) Mature asci with spores among dehisced asci. (E-F, I) Free mature ascospores. (G-H) Paraphyses, note colour (arrow). (J) Mature ascus with ascospores, showing a stipe and the spore arrangement (arrows). (K) Ascospores, note the arrangement (arrow). Scale bars: (A, C-K) = 20 µm, (B) = 50 µm.

Figure 4.4 *Saccobolus infestans* (KWSNNP018-2009).

Saccobolus 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 (Brummelen van, 1967; Doveri, 2004). *Saccobolus infestans* observed only once in a single dung type, appears to be a rare late sporulating species from Kenyan wildlife. This is a new record for Kenya.

4.3.5 ***Saccobolus platensis*** Gamundí & Ranalli, Nova Hedwigia 17: 385 (1969). (Figure 4.5 A-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-grayish 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 van Brummelen pattern I; clusters compact, 35–41 × 12–16 µm, surrounded by a hyaline, common broad gelatinous sheath.



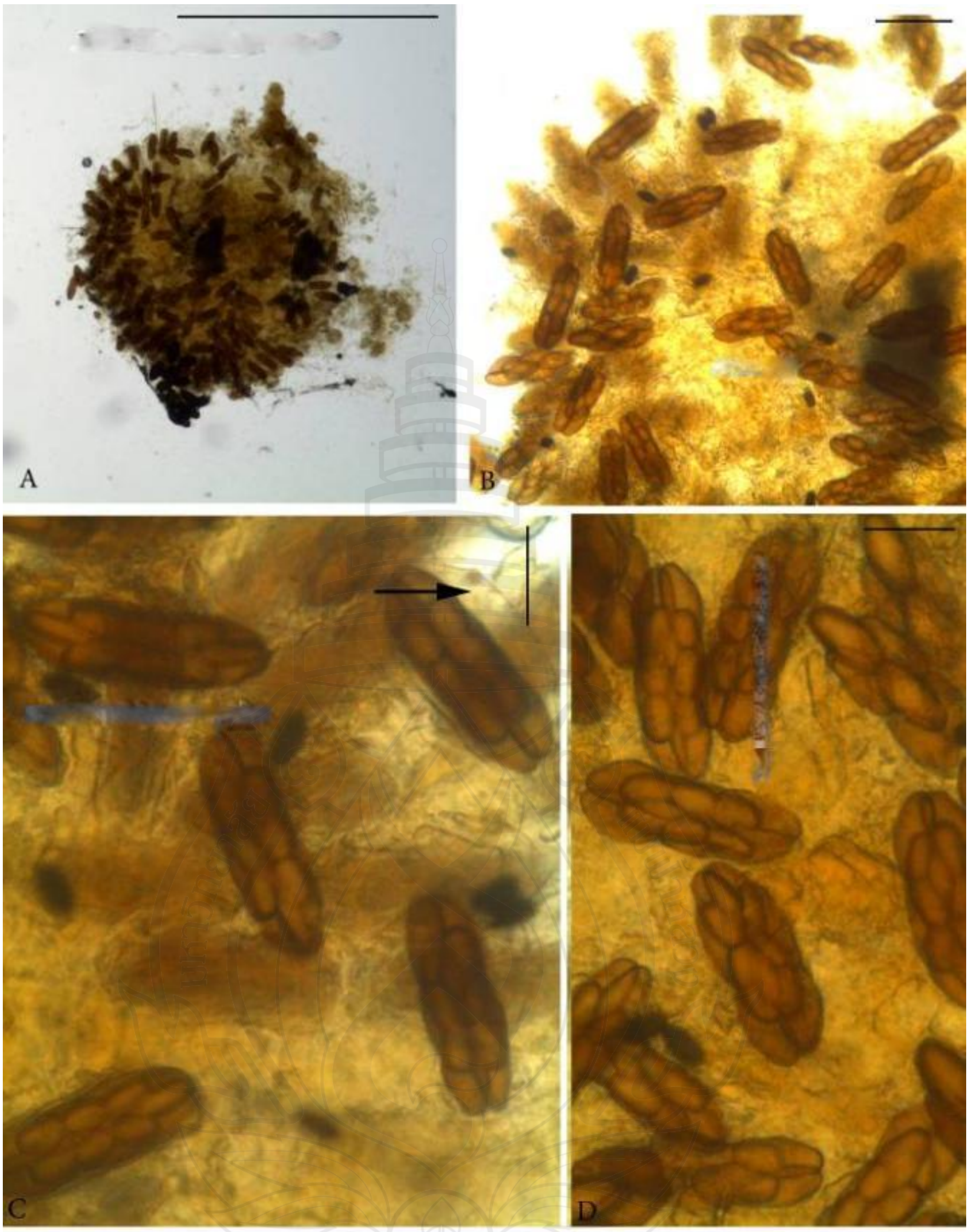
Note. (A) Squashed ascoma. (B) Details of ectal excipulum. (C) Mature asci with ascospores, note open operculum (arrow). (D) Ascospore showing spore arrangement and gelatinous sheath (arrow). (E) Ascus, note long stipe (black arrow) and closed operculum (red arrow). (F) Asci and paraphyses. Scale bars: (A) = 200 µm, (B, D-E) = 20 µm, (C, F) = 50 µm.

Figure 4.5 *Saccobolus platensis* (KWSTE005A-2008).

Saccobolus platensis is very similar to *S. minimus* from which it is differentiated by its smaller ascospores and asci and also the irregular and rough episporium (Richardson & Watling, 1997; Doveri, 2004). This was a new record for Kenya.

4.3.6 ***Saccobolus truncatus*** Vel., Monogr. Discom. Boh. 1: 370 (1934). (Figure 4.6 A-D)

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* cylindric-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, cylindric-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, episporium 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.



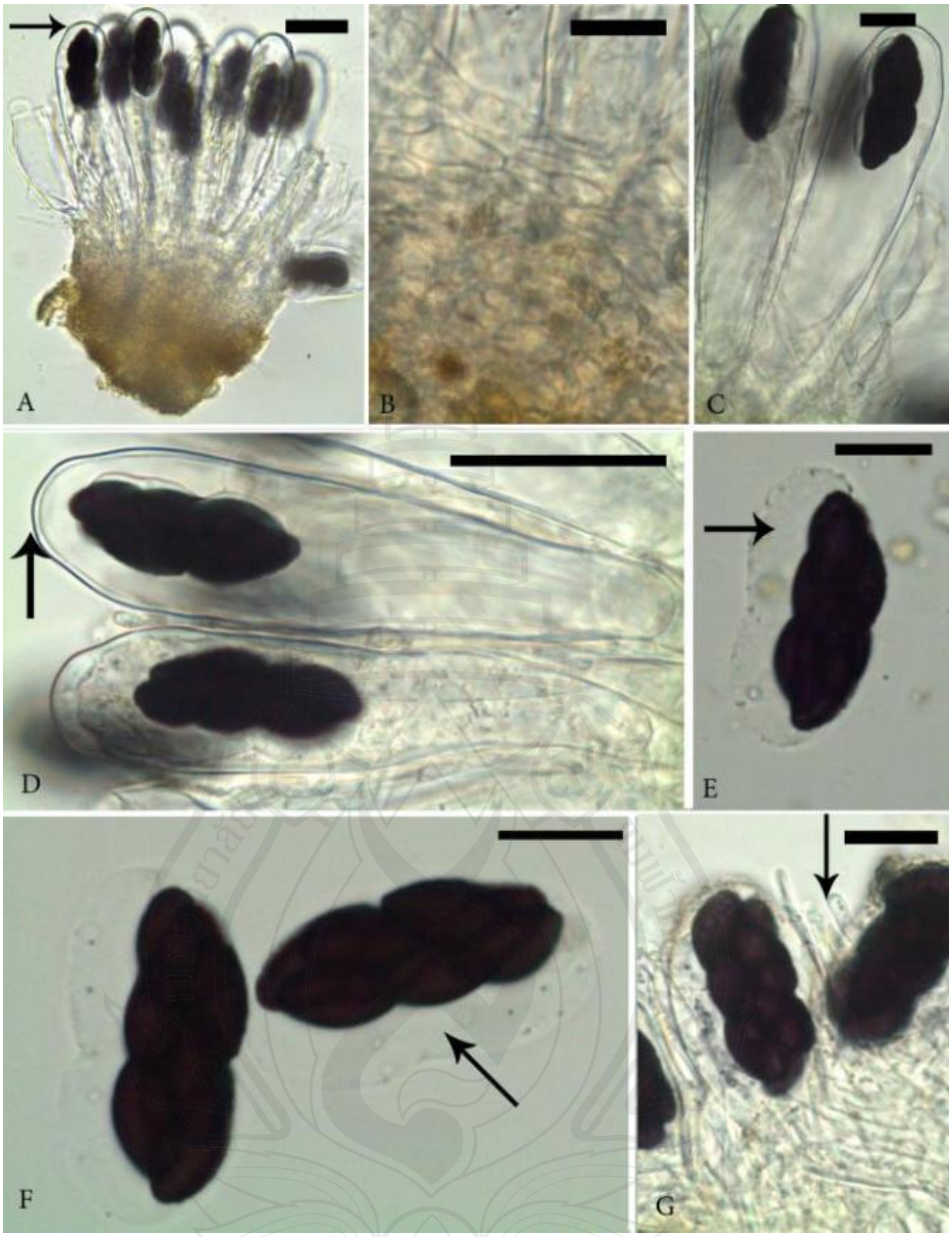
Note. (A) Squashed ascoma. (B-D) Mature ascospores, note broad hyaline gelatinous envelope (arrow). Scale bars: (A) = 200 µm, (B) = 50 µm, (C-D) = 20 µm.

Figure 4.6 *Saccobolus truncatus* (KWSTE002A-2008).

Saccobolus truncatus Sect. *Saccobolus* is related to S. citrinus and *S. minimus* (Brummelen van, 1967; Doveri, 2004). The former is differentiated from the latter by its smaller dotted ascospores and paler receptacles (Brummelen van, 1967; Doveri, 2004). The ascospores of *Saccobolus truncatus* contract characteristically at maturity (Brummelen van, 1967; Doveri, 2004). This was a new record for Kenya.

4.3.7 *Saccobolus versicolor* (P. Karst.) P. Karst., Acta Soc. Fauna Flora Fenn. 2(no. 6): 123 (1885). (Figure 4.7 A-G)

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-gray, 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* cylindric-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, episporium 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.



Note. (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 µm, (B-G) = 20 µm.

Figure 4.7 *Saccobolus versicolor* (KWSNNP020-2010).

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

4.4 Conclusions

Seven coprophilous *Saccobolus* species namely *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 common zebra dung. Five taxa, *Saccobolus citrinus*, *S. diffusus*, *S. infestans*, *S. platensis* and *S. truncatus* are new records for Kenya. The most common taxa are *Saccobolus depauperatus* and *S. citrinus*.

CHAPTER 5

COPROPHILOUS ASCOMYCETES: III. *Podospora* Ces.

5.1 Introduction

Podospora is one of the commonest genera on dung (Lundqvist, 1972; Bell, 2005; Doveri, 2008). *Podospora* in the subfamily Podosporoideae N. Lundq. (Lasiosphaeriaceae Nannf., Sordariales Chadefaud ex D. Hawksworth & O.E. Eriksson) is characterized by perithecioid, non-stromatic, more or less membranaceous, dark, superficial to semi-immersed ascomata, clavate to saccate, 4- to poly-spored asci, and ascospores 2-celled at maturity with a dark pigmented upper cell, a hyaline lower pedicel and gelatinous equipment usually as caudae. *Podospora* is strikingly similar to *Schizothecium* Corda, *Arnium* Nitschke ex G. Winter, *Zygopleurage* Boedijn and *Cercophora* Fuckel. *Schizothecium* is differentiated from *Podospora* by having perithecia with swollen agglutinated hairs particularly gathered at the neck base (Lundqvist, 1972; Doveri, 2008). Unlike *Podospora*, the immature ascospores of *Arnium* are ellipsoidal or fusiform while the mature ones are non-pedicellate (Lundqvist, 1972; Bell, 2005).

Zygopleurage differs from *Podospora* by having ascospores with two opposite dark cells connected by a long cylindrical intercalary cell. *Cercophora* has hyaline vermiform-sigmoid or cylindric-geniculate ascospores and usually cylindric-claviform asci with a thickened apical ring and often with sub-apical globules (Lundqvist, 1969, 1972; Abdullah & Rattan, 1978; Doveri, 2008). Owing to its ecology and characteristics *Podospora* has attracted the interest of many researchers over the years (Cain, 1934; Mirza & Cain, 1969; Lundqvist, 1972; Doveri, 2004, 2008; Bell, 2005; Chang & Wang, 2005).

The objectives of this survey were to examine the taxonomy of *Podospora* from Kenyan wildlife dung, to document its ecology and biodiversity on different wildlife dung types and to create awareness on the importance of dung fungi in biodiversity conservation and management.

5.2 Materials and Method

Twenty-one dung samples were cultured by moist chamber method at the KWS laboratory as described in Section 2.2 of Chapter 2. Fruiting bodies were squashed in distilled water to prepare slide mounts. Lactophenol cotton blue and Congo red stains were added on the slide mounts in order to highlight some important features that were critical in the diagnosis of Podospora species. The *Podospora* species descriptions were made based on morphological characters as detailed in monographs and dichotomous keys on members of the genus *Podospora* (Lundqvist, 1972; Bell, 1983, 2005; Richardson & Watling, 1997; Doveri, 2004, 2008).

5.3 Results and Discussion

The morphological of peridial hairs, peridium, asci and ascospores have been extensively used in generic delimitation (Cain, 1934; Mirza & Cain, 1969; Lundqvist, 1972; Bell & Mahoney, 1995; Doveri, 2004, 2008; Chang & Wang, 2005). Significant importance for the circumscription of *Podospora* species has been placed on the shape and size of the perithecium and peridial wall cells, the number of ascospores per ascus and the shape of ascospore primary and secondary appendages (Cain, 1934; Mirza & Cain, 1969; Lundqvist, 1972; Doveri, 2004, 2008; Bell, 2005; Chang & Wang, 2005). The presence or absence of hairs or bristles on the perithecia, such as those found on *Podospora anserina*, *P. australis* and *P. minor,* is another useful tool in species delimitation (Mirza & Cain, 1969; Lundqvist, 1972; Doveri, 2004, 2008; Bell, 2005). Lundqvist (1972) recognized four sections in *Podospora* namely:

1) *Malinvernia* Rab., noted for having non-pseudo-bombardioid peridium, immature ascospores dumb-bell shaped or clavate, ascospores with relatively complex caudae

2) *Andreanszkya* Toth, with 3-layered pseudoparenchymatous peridium, whose ascospores have four upper germ pores and frequently lack a pedicel

3) *Podospora* (Ces) with tough pseudo-bombardioid peridium, asci with a thin apical ring and

4) *hypophila* Lundq., recognized by perithecia with black tubercles on base of neck and its lack of an apical ring on ascus, fibrillate or lamelate caudae and frequent polyspory

Cultural attributes, though not extensively studied, seem to be important in identifying *Podospora* species and groups of related taxa. *Podospora,* a cosmopolitan and usually coprophilous genus was treated in detail by Cain (1934), Mirza & Cain (1969), Lundqvist (1972), Bell & Mahoney (1995), Bell (2005) and Doveri (2008). Detailed descriptions of *Podospora* species examined are as presented in section 5.3.1 to 5.3.5.

5.3.1 *Podospora anserina* (Ces. ex Rabenh.) Niessel, Hedwigia 22:156, 1883. (Figure 5.1 A-K)

Ascomata perithecioid, semi-immersed to nearly superficial, 380–600 µm high, 300–500µm diam., scattered or in small groups, covered with few black tubercles and numerous hyphoid hairs, conical or pyriform; *neck* subcylindric, 90–120 µm × 70–80 µm, blackish, coriaceous, with few tufts of agglutinated rigid straight, brown, pointed hairs, 100–300 µm long, continuous or sparingly septate. *Peridium* pseudoparenchymatous: endostratum of pale translucent thin-walled polygonal cells 10–25 µm wide; exostratum of thick-walled polygonal cells where hyaline wavy septate hairs originate, 2–4 µm wide; *Paraphyses* cylindric-moniliform, exceeding the asci, 4.5–6.5 µm broad, septate, with hyaline vacuoles. *Asci* 4-spored, 175–275 × 22–29 µm, clavate, with a thin apical ring, long and slender, lobate stipe. *Ascospores* obliquely uniseriate, two-celled at maturity: spore head 32–36 × 17–20 µm, immature spoon shaped, mature ellipsoidal, somewhat unequilateral, smooth, dark brown, with a centric germ pore; lower cell (pedicel) cylindrical, 20–30 × 4–5 µm, slightly pointed; *upper cauda*, sub-apical, fairly long, lash-like, furrowed, tip curved, 30–80 × 6.5–8 µm at base; *lower cauda* solid, filiform; additional two to three, short, filiform caudae, with hooked tips, attached to basal part of primary appendage near septum.

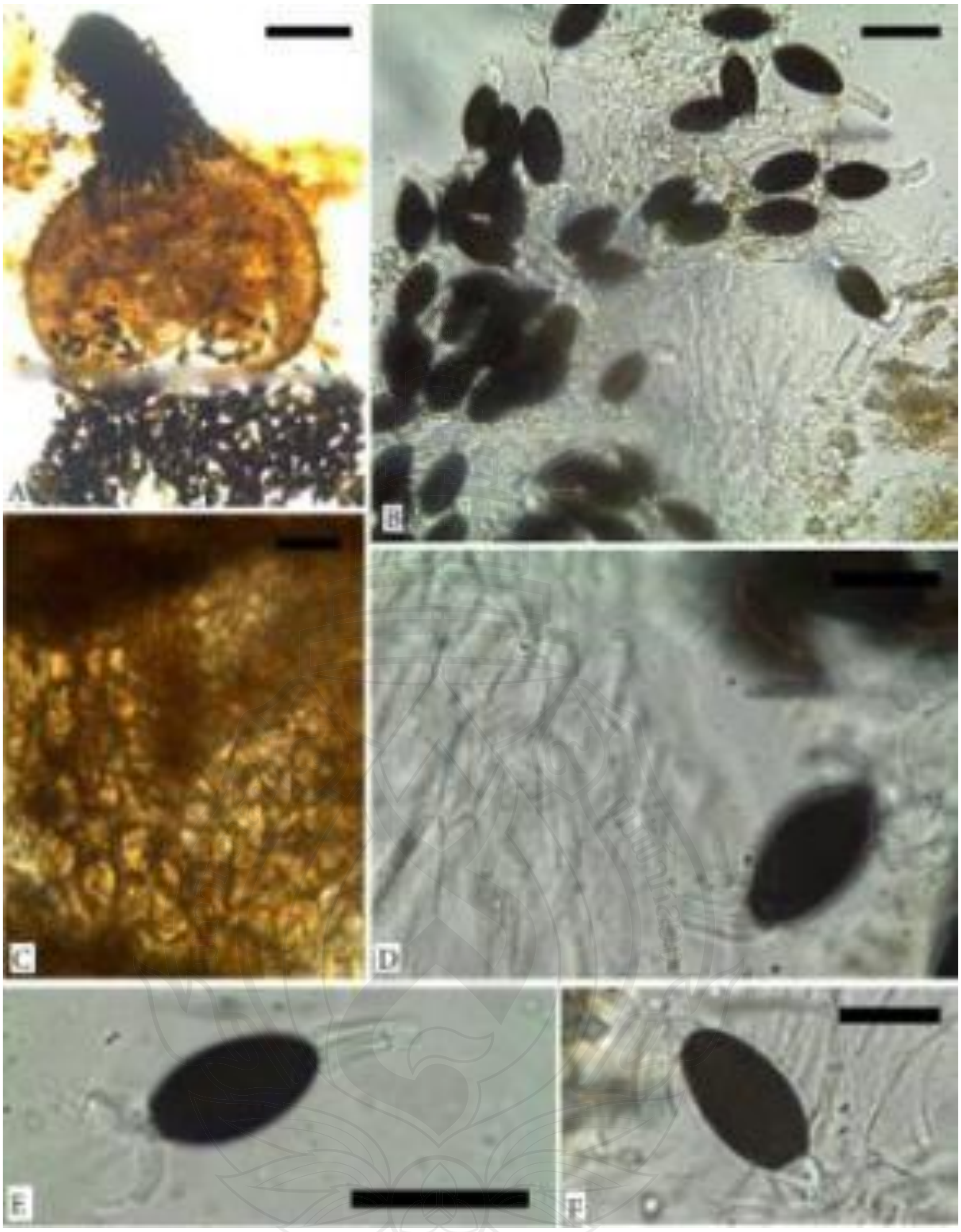
Note. (A) Ascomata on dung, note tufts of pointed hairs (arrows). (B) Whole ascoma water mount, note hairs (arrows). (C-D, G-H) Mature ascospores showing pedicels and caudae (arrows). (E) Asci and ascospores showing uniseriate spore arrangement. (F) Squashed ascoma. (I) Paraphyses, note septation (arrow). (J) Ascus showing stipe (arrow). (K) Details of exoperidium. Scale bars: (A-B) = 200 µm, (C-D, H-K) = 20 µm, (E, G) = 50 µm, (F) = 500 µm.

Figure 5.1 *Podospora anserina* (KWSANP004-2009).

Podospora anserina Sect. *Malinvernia* Rabenh. is distinguished from *P. australis* (Speg.) Niessl Sect. *Andreanszkya* Tóth. by the former's smaller non-apiculate ascospores with more complex furrowed caudae and the latter's notably simpler taenioid caudae (Lundqvist, 1972; Doveri, 2004, 2008; Bell, 2005). The morphology of our collection agrees with that observed for this taxon in previous examinations (Cain, 1934; Lundqvist, 1972; Bell, 1983, 2005; Doveri, 2008). This is a fairly common species on wildlife dung in Kenya.

5.3.2 *Podospora argentinensis* (Speg.) J.H. Mirza & Cain, Can. J. Bot. 47: 2008, 1969. (Figure 5.2 A-F)

Ascomata perithecioid, immersed or semi-immersed, 400–670 µm high, 200–500 µm diam., scattered, upper portion of perithecia with black tubercles, globose to pyriform. *Peridium* membranaceous; endoperidium of thin-walled polygonal cells; exoperidium composed of brownish thin translucent *textura globulosa-angularis*, cells measuring 6–11 × 5–7 µm. *Neck* short cylindrical, fully covered with black papillae. *Paraphyses* numerous, filiform above, ventricose below, fugacious. *Asci* 8-spored, 180–200 × 30–40 µm, clavate, narrowly rounded at apices, long stipitate. *Ascospores* biseriate, two-celled: spore head 27.5–35 × 15–19 µm, ovoid-ellipsoidal, smooth, brown to blackish, thin walled, with an eccentric germ pore; pedicel often appearing twisted, flattened, cylindrical, or slightly clavate 18–23 µm long, 6.5–9 µm broad at base; upper caudae formed as a lyre-shaped structure, sometimes elongated; lower caudae, fugacious, small, formed as a whorl at the proximal end of the pedicel.



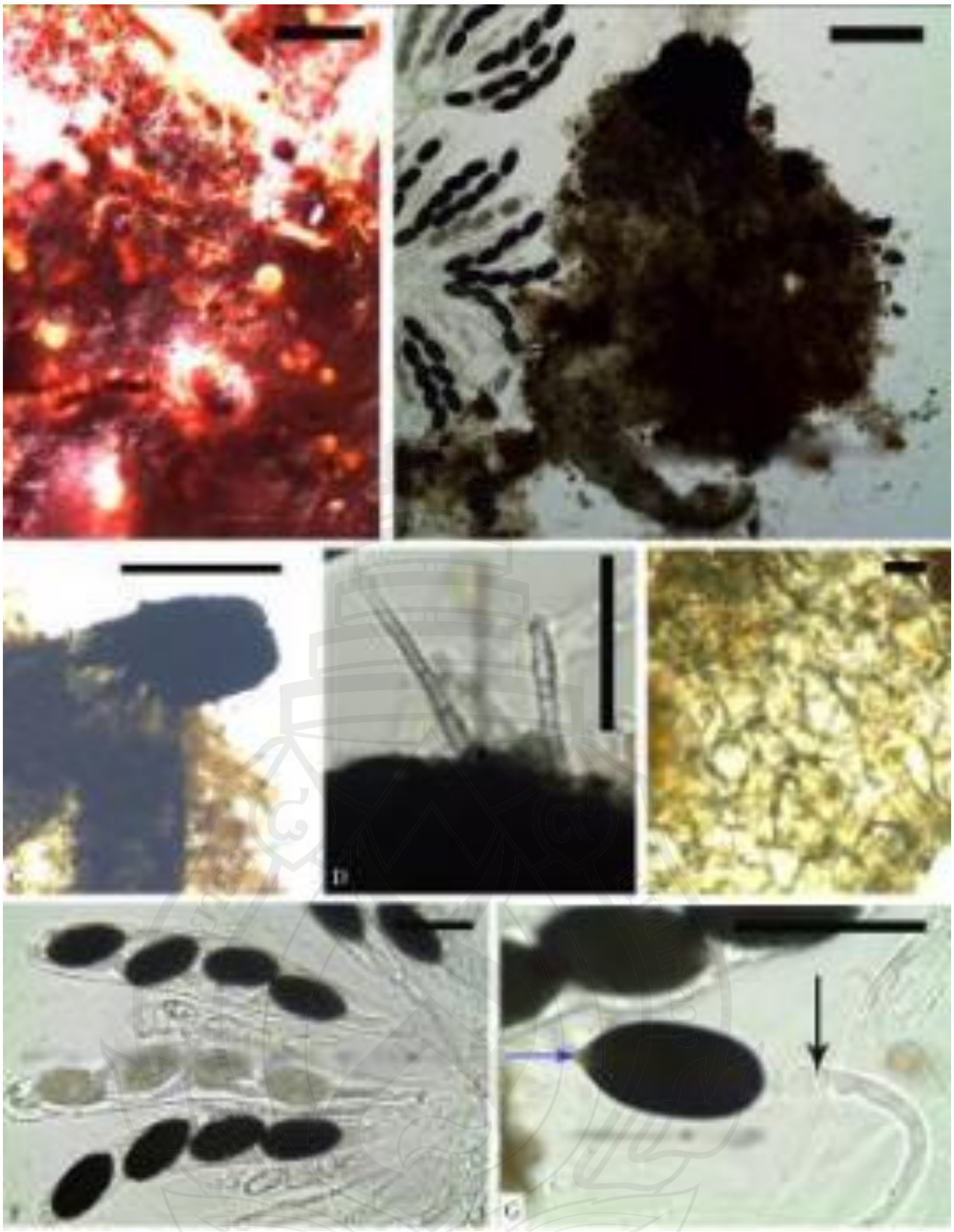
Note. (A) Ascoma squash. (B) Free mature ascospores. (C) Details of exoperidium. (D) Paraphyses. (E-F) Free mature ascospores showing pedicel and caudae. Scale bars: (A) = 200 µm, (B) = 50 µm, (C-F) = 20 µm.

Figure 5.2 *Podospora argentinensis* (KWSACC002-2009).

Podospora argentinensis sect. *Rhypophila* Lundq. is closely related to *P. decipiens* (G. Winter ex Fuckel) Niessl of the same section but the former has smaller ascospores (Mirza & Cain, 1969; Bell, 2005) while the latter does not have a cauda attached at the end of the pedicel (Chang & Wang, 2004). This species has been recorded many times from tropical areas and is deemed to be the tropical region's substitute of *P. decipiens* (Mirza & Cain, 1969; Lundqvist, 1973; Krug & Khan, 1989; Richardson, 2001). The characters of the Kenyan collection are similar to the descriptions made in previous studies (Mirza & Cain, 1969; Bell, 2005).

5.3.3 *Podospora australis* (Speg.) Niessl, Hedwigia 22: 156, 1883. (Figure 5.3 A-G)

Ascomata perithecioid semi-immersed to superficial, 550–850 µm high to 500–620 µm diam., scattered, olive brown to nearly black, pyriform; the exposed portion covered with long, septate, branched, olivaceous brown and hyaline tipped hairs 2.5–3 µm broad. *Peridium* semi-membranaceous, translucent to slightly opaque; endoperidium of *textura angularis* cells; exoperidium of thick-walled *textura angularis* cells 8–11 × 6.5–8.5 µm. *Neck* 180–240 × 180–190 µm, black, opaque, papilliform to cylindrical; with, septate, rigid hairs 27.5–38.5 µm long, 2–4 µm broad at base. *Paraphyses* cylindric-moniliform or filiform, longer than asci, 5–6 µm broad, septate, rarely branched. *Asci* 4-spored, 258–307 × 36.5–44 µm, unitunicate, narrowly cylindrical, tapering below into a slender very long stipe, with an indistinct apical apparatus, apex narrow. *Ascospores* obliquely uniseriate at maturity, two-celled: spore head 50.5–55.5 × 25–27 µm, narrowly ovoid to ellipsoidal, symmetrical, dark brown, smooth, thick walled, flattened at base with an apical conspicuous germ pore; lower cell (pedicel) reduced to a conspicuous small basal triangular apiculum, hyaline, 4–6.5 × 2–3 µm; *upper cauda* 50–100 µm long, 8–15 µm broad at the base, more segmented (taenioid) in proximal part, longitudinally furrowed and poly-channeled, wavy, attached slightly eccentrically, not covering the germ pore; *lower cauda* similar, long, originating from the basal end of spore, completely covering the apiculum, hollow and taenioid.



Note. (A) Necks of semi-immersed ascomata on dung. (B) Ascoma squash. (C) Perithecial neck. (D) Septate and hyaline perithecial hairs. (C) Details of exoperidium. (F) Mature and immature asci. (G) Free mature ascospore showing taenioid caudae (black arrow) and apiculum (blue arrow). Scale bars: (A) = 1000 µm, (B-C) = 200 µm, (D-E, G) = 20 µm, (F) = 50 µm.

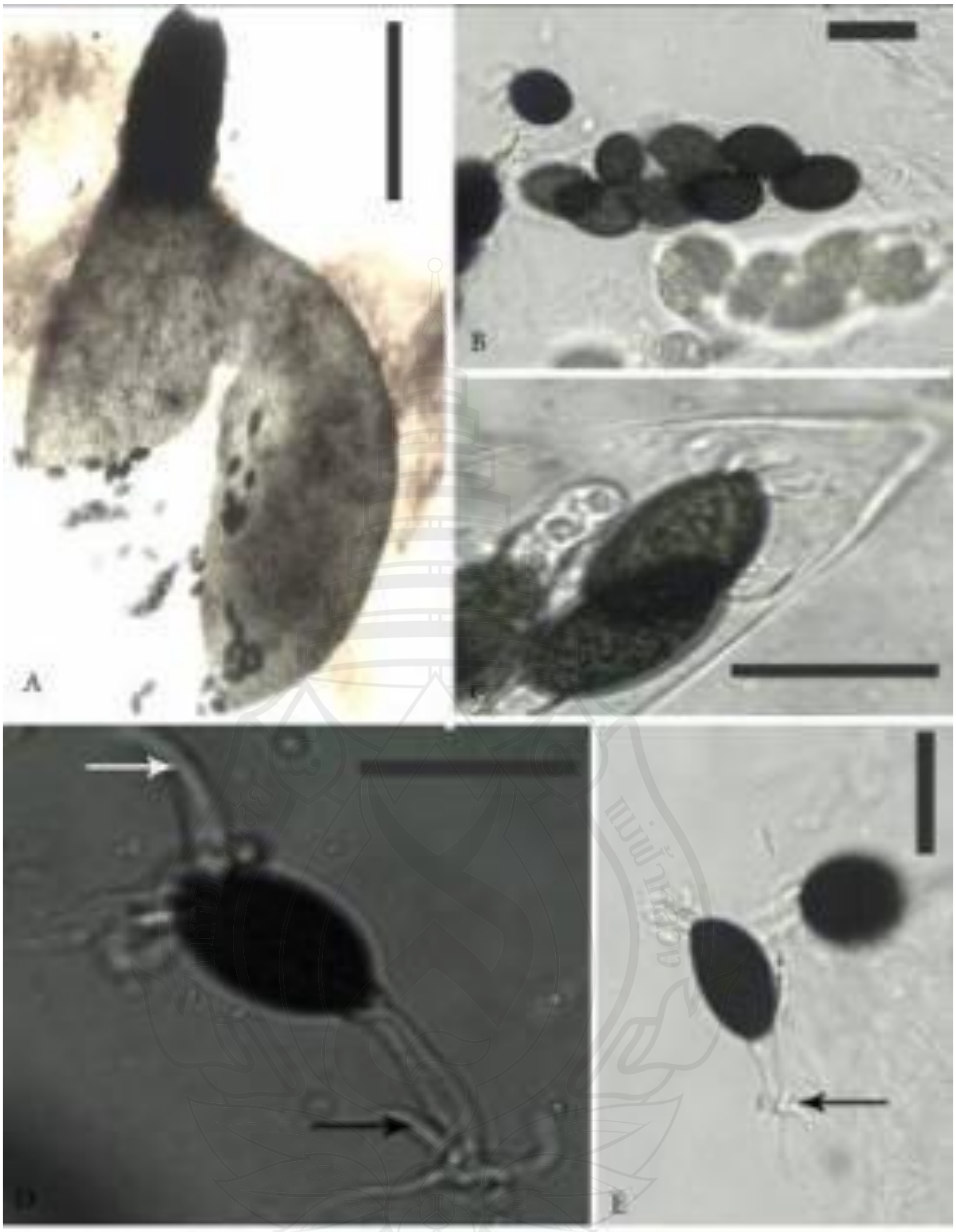
Figure 5.3 *Podospora australis* (KWSACC002-2009).

Podospora australis Sect. *Andreanszkya* (Tóth) Lundq., noted for having a reduced pedicel, is quite unique and is identified by the large ascospores with a small, basal apiculum and taenioid caudae, one on each side of the spore head (Mirza & Cain, 1969; Lundqvist, 1972; Doveri, 2004, 2008; Bell, 2005). It is differentiated from the related *P. anserina* by its larger apiculate ascospores and longitudinally poly-channeled and transversely banded caudae (Lundqvist, 1972; Bell, 2005). Lundqvist, (1972) argues that *Podospora apiculifera* (Speg) Mirza & Cain is a depauperate form of *Podospora australis*. Previous observations of this taxon in Kenya were made on dung of steenbok, impala, bushbuck, hippopotamus, buffalo, zebra, eland, giraffe and cattle (Krug & Khan, 1989; Caretta et al., 1998). It seems to be a very common species on wildlife dung.

5.3.4 ***Podospora communis*** (Speg.) Niessl, Hedwigia 22:156,1883. (Figure 5.4 A-E)

Ascomata perithecioid, superficial, 670–1100 × 410–530 µm, scattered or gregarious in small groups, semi-transparent, becoming olivaceous or dark brown, adorned with fine brown hairs of over 40 µm long, disappearing with age, obpyriform. Neck conical or cylindrical, 150–385 × 130–150 µm, roughened with small, black papillae or glabrous, opaque, usually curved; ostiole over 80µm diameter. Peridium membranaceous, semi-transparent; endoperidium of textura angularis cells; exoperidium consisting of yellowish-olive textura angularis cells. Paraphyses reduced to a mass of elongated vesicles. Asci 240–265 × 40–50 µm, 8-spored, unitunicate, clavate, with a narrow tapering broad apex, fairly long stipe, apical ring thin and barely visible. Ascospores biseriate, two-celled: spore head 29–40 × 16–25 µm, at first sub-cylindric, later ellipsoidal with truncate base, occasionally asymmetrical, dark to olivaceous brown, opaque, thick walled, with a minute apical germ pore; pedicel 25–42 × 5–6 µm, cylindrical, wider at the base, hyaline, slightly curved in the early stages, straight later; upper caudae four, flattened, short, independent, curved, composed of two filaments, lash-like, larger, 40–50 × 2–4 µm diam., sub-apical, surrounding the germ pore; lower caudae four, 3.5–5 × 1.5–2.5 µm, curved, each independently arising from apex of the pedicel, lash-like, 3.5–5 × 1.5–2.5 µm.

Podospora communis sect. *Malinvernia* in our collection has typical features for the species and compares well with descriptions of same species in previous examinations (Lundqvist, 1972; Bell, 2005). *Podospora communis* is diagnosed by having clavate or dumb-bell shaped immature ascospores with complex gelatinous equipment at maturity, and peridium lacking a middle gelatinous layer. The main diagnostic characters for *P. communis* include its long, cylindrical pedicel equipped with four short, claw-like, independent gelatinous caudae on the lower end and the presence of four similar caudae on the apex of the spore head (dark cell) (Lundqvist, 1972). However, our Kenyan collection has some slight variance with the Australian collection which had three basal caudae (Bell, 2005). *Podospora spinulosa* R.S. Khan & Cain, in the same section, is differentiated from *P. communis* by having short spinules on perithecial neck and more caudae at the base of the pedicel; *P. hyalopilosa* (R. Stratton) Cain has hyaline perithecial neck hairs and a pedicel with only a single apical cauda; *P. multicaudiculata* Cailleux has a slightly hairy neck, shorter asci and multiple but simpler structured caudae; *P. austrohemisphaerica* N. Lundq. has rigid neck hairs, larger spores, multiple caudae, usually four at each pole, additonal shorter caudae at base of pedicel and a sheath covering the pedicel and spore head; the neck of *P. deropodalis* R.S. Khan & Cain is covered with short spinules, it has smaller spores but with longer pecidels and variable number of upper caudae and fewer lower caudae; *P. alexandri* Doveri as well, is similar but can be differentiated by its larger ascospores and the inconspicuous caudae when mounted in water (Doveri 2008). *Podospora communis* is the most widespread and commonest occurring on several wildlife dung types in Kenya.

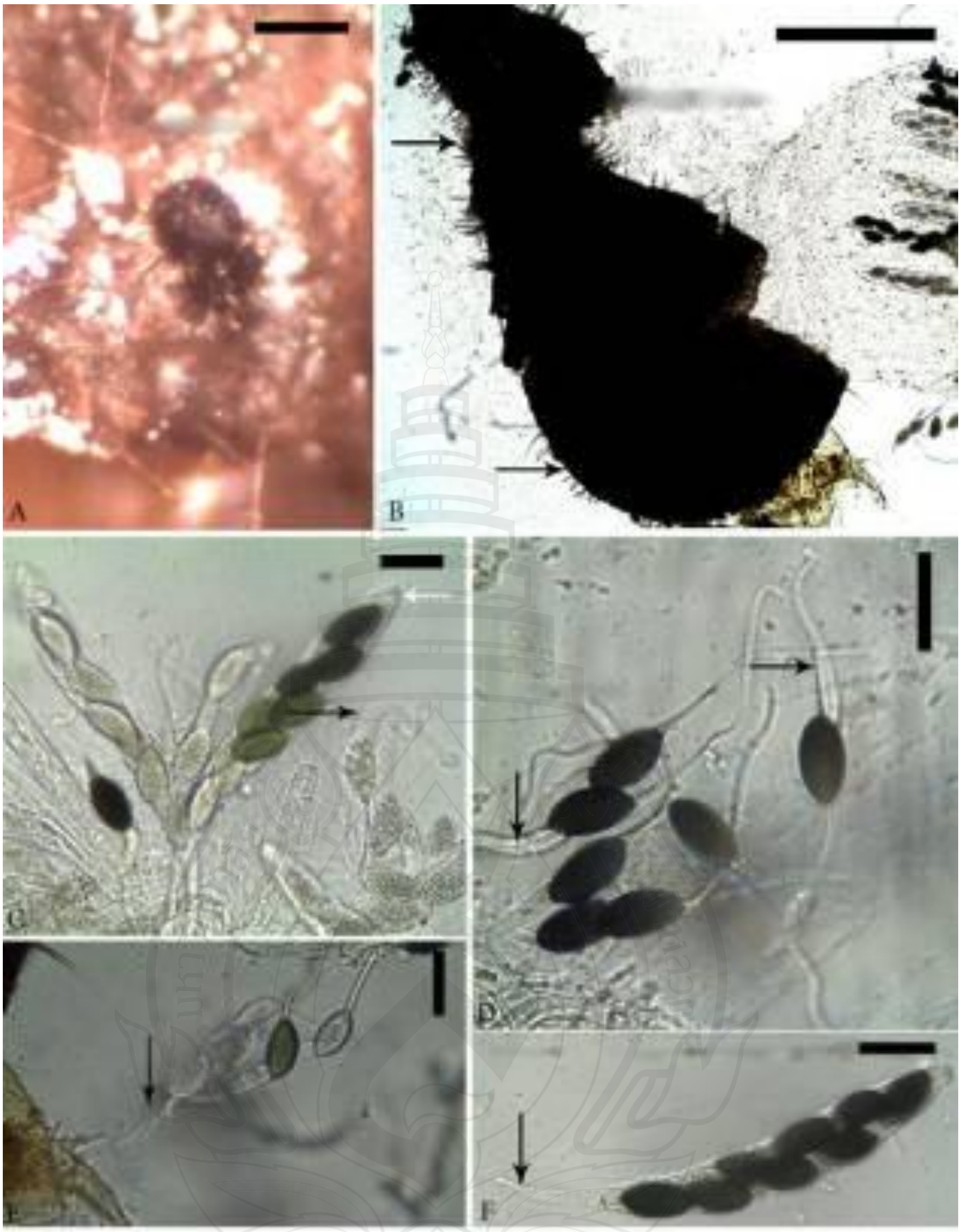


Note. (A) Ascoma squash. (B) Asci with ascospores in different stages of maturity. (C) Pointed ascus tip. (D) Free mature ascospore showing 4 apical (white arrow) and 4 basal caudae (black arrow). (C) Free mature ascospore showing 4 basal caudae (arrow). Scale bars: (A) = 200 um, (B, E) = 50 um, (C-D) = 20 um.

Figure 5.4 *Podospora communis* (KWSNNP001-2009).

5.3.5 *Podospora minor* Ellis & Everh., Amer. Nat. 31: 341, 1897. (Figure 5.5 A-F)

Ascomata perithecioid, superficial, 450–810 µm high., 165–470 µm diam., scattered, black, opaque, coriaceous, with short rigid septate, brown and hyaline tipped hairs 23.5–53 × 3–4 µm, conical to pyriform. *Neck* black, cylindrical, 150–200 × 200–225 µm, adorned with stiff septate hyaline tipped hairs. *Peridium* coriaceous, pseudo-bombardioid, 4-layered; outermost peridial wall layer composed of *textura angularis* thick-walled brown elongated polygonal cells; second layer from outside made up of hyaline, thick-walled gelatinous polygonal cells; third layer of darker, thick-walled flattened cells; fourth layer composed of lighter thin-walled polygonal cells. *Paraphyses* exceeding the asci, filiform above and ventricose below, septate, 3–4.5 µm broad, hyaline. *Asci* 8-spored, 245–345 × 27.5–43 µm, cylindrical-clavate, unitunicate, narrow, evanescent, round apex, short stipitate. *Ascospores* uniseriate to biseriate, at first hyaline, single-celled, fusiform then cylindrical; spore head 37.5–47.5 × 20.5–25 µm at maturity, slightly inequilateral, black-brown, ellipsoidal to fusiform; germ pore apical; pedicel hyaline, cylindrical or clavate, broader at the tip; upper cauda longer and broader, 75.5–126.5 × 9–12 µm, lash-like, appearing striated and furrowed, eccentric, not covering the germ pore, hollow; lower cauda shorter and narrower, 46–73.5 × 4.5–5 µm, hyaline, hollow, attached to distal end of the pedicel, tips circinate.



Note. (A)Ascoma on dung. (B) Squashed ascoma mount, note hairs (arrow). (C) Mature and immature asci and ascospores, note apex (white arrow) and gelatinous equipment (black arrow). (D-E) Free mature and immature ascospores, note caudae with circinate ends (arrows). (F) Ascus, showing spore arrangement and stipe (arrow). Scale bars: (A) = 500 µm, (B) = 200 µm, (C-F) = 50 µm.

Figure 5.5 *Podospora minor* (KWSACC002-2009).

Podospora minor in the section *Podospora* has ascospores that are morphologically similar to those of *P. fimiseda* (Ces. & De Not.) Niessl. belonging to the same section but are slightly smaller. The characters of *Podospora minor* are intermediate between *P. appendiculata* (Auswer.) Niessl and *P. fimiseda*. Our collection has broader asci and larger ascospores in comparison to the specimen examined by Mirza & Cain (1969).

5.4 Conclusions

Ten dung types produced twenty-eight specimens of *Podospora*. From these specimens, five species namely, *Podospora anserina*, *P. argentinensis*, *P. australis*, *P. communis* and *P. minor* were described and illustrated. *Podospora minor* seems to be a rare species and is recorded for the first time in Kenya. *Podospora communis*, *P. anserina* and *P. australis* are the most common species on dung types examined.

CHAPTER 6

COPROPHILOUS ASCOMYCETES: IV. *Schizothecium* Corda, Emend. Lundq.

6.1 Introduction

The cosmopolitan and usually coprophilous genus *Schizothecium* belongs to the family Lasiosphaeraceae (Lundqvist, 1972; Doveri, 2008) and is related to *Podospora*, *Arnium*, *Zygopleurage* and *Cercophora* (Lundqvist, 1972; Hu et al., 2006; Doveri, 2008). The perithecia are usually covered with hyphoid hairs. Asci are always poly-spored, cylindrical to clavate and often lacking an apical ring. The asci may be enveloped by moniliform paraphyses. The usually uni- to multi-seriate ascospores are 2-celled when mature and undergo an early transverse septation, have a plasma-filled, usually cylindrical and persistent hyaline pedicel, with a single germ pore and normally a thin gelatinous sheath and/or solid lash-like caudae (Lundqvist, 1972; Bell & Mahoney, 1995; Hu et al., 2006; Doveri, 2008).

Schizothecium has been recorded in Europe (Caretta et al., 1994), Asia (Furuya & Udagawa, 1972), Americas (Richardson, 2008) and Africa (Krug & Khan, 1989). It has also been recorded from wild herbivore dung in Kenya (Caretta et al., 1998).

This study sought to document the taxonomy of *Schizothecium* species found on various dung types in Kenyan wild herbivores and their diversity and distribution on different dung types.

6.2 Materials and Methods

Wildlife dung was incubated in moist chambers as described in section 2.2 of Chapter 2 and the *Schizothecium* fruiting bodies seen and isolated were subjected to the procedures described in Sections 2.2.3 and 2.2.4 of Chapter 2. The *Schizothecium* species described in this work were identified based on morphological characters as described in monographs and dichotomous keys on members of the genus (Mirza & Cain, 1969; Lundqvist, 1972; Bell, 1983, 2005; Richardson & Watling, 1997; Doveri, 2008).

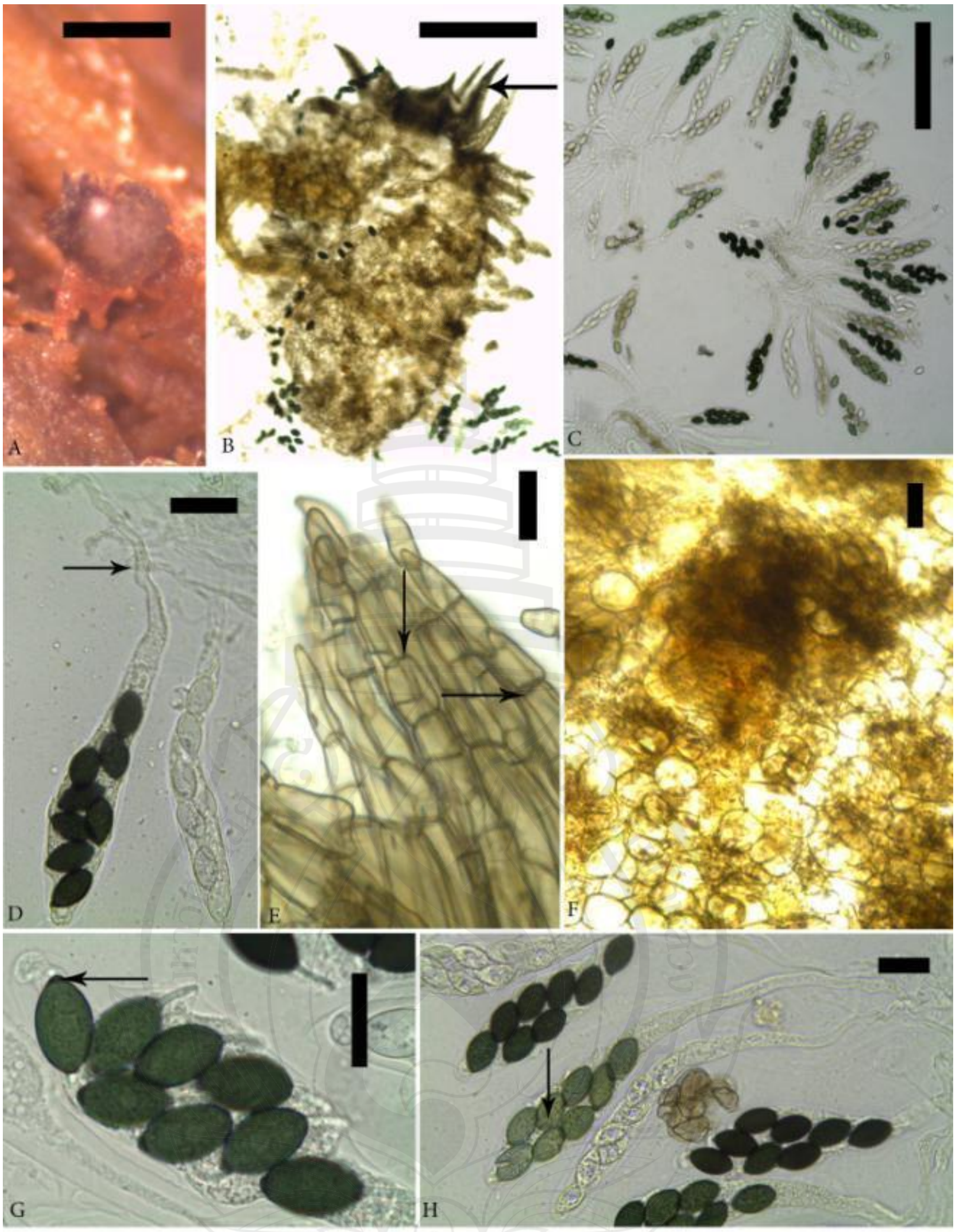
6.3 Results and Discussion

Schizothecium in Podosporoideae N. Lundq. (Lasiosphaeriaceae Nannf.) is differentiated from the closely related *Podospora*, by having ascomata with either agglutinated or sometimes isolated, articulated or one-celled, swollen hairs, often in a palisade of triangular scales at the base of the perithecial neck. *Arnium* Nitschke ex G. Winter is differentiated by having non-pedicelate spores while *Cercophora* Fuck. emend. Lundq. has spores with sigmoid pedicels (Lundqvist, 1972; Cai et al., 2005; Hu et al., 2006; Doveri, 2008). The perithecia are often covered with hyphoid hairs and the peridium is membranaceous, pseudoparenchymatous, composed of a *textura globulosa* or *globulosa-angularis* (Lundqvist, 1972; Doveri, 2008). Asci are 4- to poly-spored, cylindrical to clavate and often lacking an apical ring. Unlike these related genera, true paraphyses that mix with asci are absent. Instead, asci are usually surrounded by broad, moniliform paraphyses [“jacket” paraphyses according to Bell & Mahoney (1995)]. The uni- to multi-seriate ascospores are 2-celled at maturity after undergoing an early transverse septation, have a plasma-filled, usually cylindrical and persistent hyaline pedicel, with a single germ pore and normally a thin gelatinous sheath and/or solid lash-like caudae. Mirza & Cain (1969), Lundqvist (1972), Bell & Mahoney (1995) and Doveri (2008) made very detailed studies on this genus.

Molecular studies (Hundhorf et al., 2004; Cai et al., 2005; Cai et al., 2006) have improved the knowledge of this genus and contributed to better circumscription of species putting to rest decades of debate on whether to treat this genus as distinct from *Podospora* (Lundqvist, 1972; Krug & Khan, 1989; Bell & Mahoney, 1995; Doveri, 2008). *Schizothecium* species from Kenyan wildlife dung are described and illustrated in the sections below.

6.3.1 *Schizothecium conicum* (Fuckel) N. Lundq., Symb. Bot. Upsal. 20 (no. 1): 253 (1972). (Figure 6.1 A-H)

Ascomata perithecioid, immersed, 890–1200 × 570–750 µm, gregarious, dark above and olivaceous-brown below, sub-pyriform-conical. *Neck* relatively short, with pronounced crown of long tufts of swollen agglutinated septate hairs with segments 26–84 × 14–28.5 µm, terminal cells more elongated and pointed, with some angular cells, black, opaque. *Peridial wall* thin membranaceous, semi-transparent, with distinct *textura globulosa* cells 18–24.5 × 16–19.5 µm. *Paraphyses* cylindric-moniliform, septate, evanescent, mixed with the asci. *Asci* 8-spored, 214–321 × 31.5–38 µm, unitunicate, cylindrical-clavate after swelling or collapsing in water mounts, with apical apparatus, broadly rounded above, with indistinct thickened apical ring, tapering below into a long stipe 36–100 × 1–4 µm. *Ascospores* dark cell 26–29 × 15–17 µm, two-celled, broadly ellipsoidal with truncate base, ranging through green, olivaceous to dark olivaceous-black in reflected light, uniseriate to biseriate or irregular in central part, pointed ends; *pedicel* cylindric, slender 12–14 × 2–3 µm, pointed and curved; *upper cauda* slightly eccentric, 30–60 × 4–6 µm, *lower cauda* central, 8.5–12 × 2–3.5 µm. *Germ pore* apical.



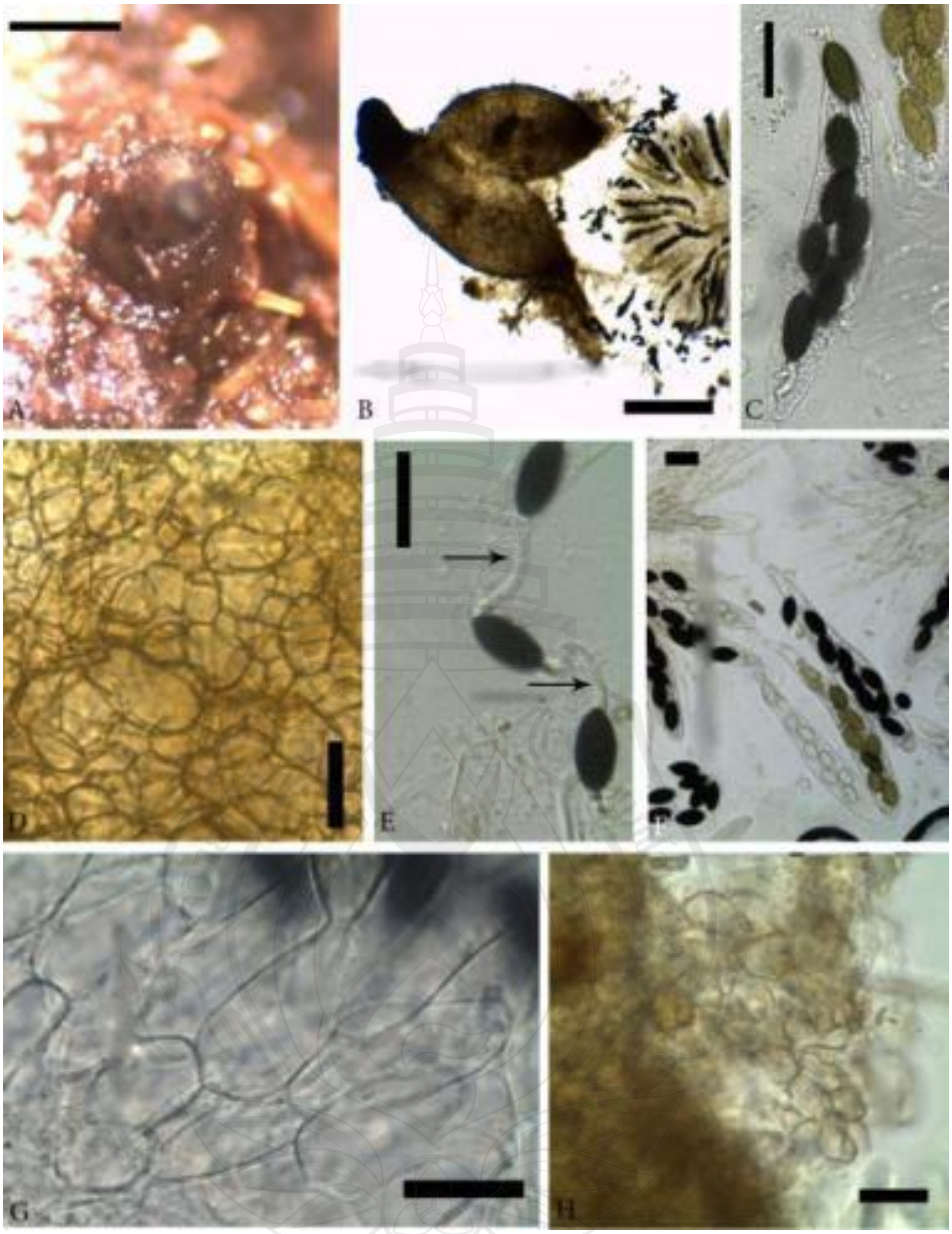
Note. (A) Ascoma on dung. B Squashed ascoma, note agglutinated hairs (arrow). (C) Asci. (D) Mature and immature ascus, note long stipe (arrow). (E) Upper part of agglutinated hairs on base of neck, note septation (arrow). (F) Details of peridial wall. (G) Mature ascospores, note germ pore (arrow). (H) Asci, ascospores and paraphyses, note biseriate spore arrangement (arrow). Scale bars: (A) = 500 µm, (B-C) = 200 µm, (D, H) = 50 µm, (E-G) = 20 µm.

Figure 6.1 *Schizothecium conicum* (KWSNNP015-2010).

Schizothecium conicum ascospores are similar to those of *S. aloides* (Fuckel) N. Lundq., but are slightly shorter (14.5–17 µm) compared to *S. aloides* (15.5–17 µm), they are almost the same breadth. *Schizothecium aloides* has longer agglutinated neck hairs. Other similar species include *Schizothecium curvuloides* (Cain) N. Lundq, *S. glutinans* (Cain) N. Lundq and *S. miniglutinans* (J.H. Mirza & Cain) N. Lundq which are described below. Our Kenyan material has ascospores that fall within the range provided from previous investigations (Mirza & Cain, 1969; Lundqvist, 1972; Bell & Mahoney, 1995; Doveri, 2008). *Schizothecium conicum* is a new record for Kenya.

6.3.2 *Schizothecium curvuloides* (Cain) N. Lundq. var**. Curvuloides**, Thunbergia 25: 10, 1996, [1997]. (Figure 6.2 A-H)

Ascomata perithecioid, superficial, 630–1100 µm high, 350–850 µm diam., scattered or in small groups, pale brown, ovate-pyriform to pyriform, membranaceous, hairy, with a black, coriaceous, sometimes curved, conical or subcylindric neck, 270–300 µm high, 210–220 µm diam., adorned at its base with few inconspicuous clusters of swollen, agglutinated, thick-walled, curved hairs, ca. 32 × 7 µm formed of 1–4 cells, terminal cells more elongate, rounded with darker end wall; long flexuous hairs covering the venter. *Peridium* layered with an exostratum composed of *textura angularis-globulosa*, thick-walled cells 9–23 × 7–14 µm. *Jacket Paraphyses* moniliform, hyaline, septate, 9.5–10 µm broad at septa, mid-section 15–20 µm broad. *Asci* 8-spored, 243–320 × 26–40 µm, cylindrical-clavate, easily swelling in water up to 70 µm diam., apex simple with a cytoplasmic plug, apical ring indistinct, with long crooked stipes. *Ascospores* uniseriate to biseriate above; spore head 35–45 × 17.5–23.5 µm, elongate-ellipsoidal, olivaceous-black, thick-walled, apex umbonate; transverse striations visible in young, scarcely pigmented spores but occasionally lacking in some collections, with an apical germ pore; *pedicel*, thin, hyaline, cylindrical, 6–9 × 2–4 µm, pointed at the apex; *upper cauda* eccentric, cylindrical, 26–60 × 5.5–7 µm, with few grooves on one side; *lower cauda* arising from the distal end of pedicel, lash-like, 26–50 × 1.5–2 µm broad at the base.



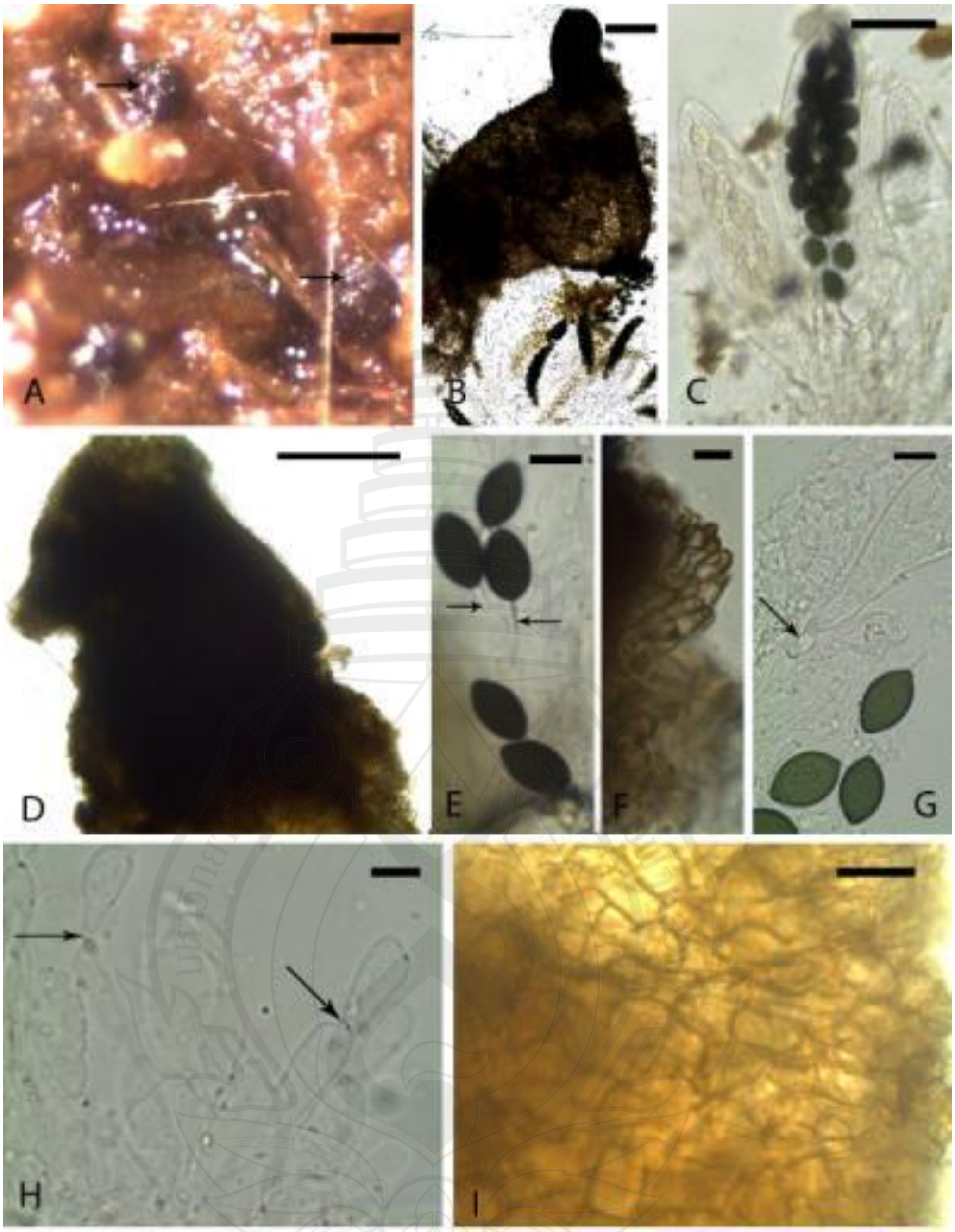
Note. (A) Ascoma on dung. (B) Squashed ascoma (C) Mature ascus. (D) Details of exoperidial wall. (E) Mature ascospores showing caudae (arrows). (F) Immature and mature asci. (G) Jacket paraphyses. (H) Agglutinated hairs. Scale bars: (A-B) = 500 µm, (C, E-F) = 50 µm, (D, G-H) = 20 µm.

Figure 6.2 *Schizothecium curvuloides* var. *curvuloides* (KWSNNP008-2009).

Schizothecium curvuloides var. *curvuloides* resembles *S. glutinans*. It is, however, differentiated from the latter by having narrowly rather than broadly ellipsoidal, transversely striated ascospores with shorter pedicels (Cain, 1934; Bell & Mahoney, 1995; Doveri & Coué, 2008a). *Schizothecium curvuloides* var. *curvuloides* is easily confused with the closely related *S. curvuloides* var. *megasporum* Doveri & Coué but the latter can be differentiated by having larger perithecia, asci, and spore heads lacking transverse striations (Doveri & Coué, 2008a). *Schizothecium curvuloides* var. *curvuloides* is fairly common on wildlife dung in Kenya.

6.3.3 *Schizothecium dakotense* (Griffiths) N. Lundq., Symb. Bot. Upsal. 20 (1): 254, 1972. (Figure 6.3 A-I)

Ascomata perithecioid, immersed to nearly superficial, 520–750 µm high, 120–200 µm diam., scattered, olivaceous brown, elongate-pyriform, with short, blackish, sometimes curved swollen hairs, scattered all over the venter. *Neck* curved, black, subcoriaceous, conical, 140–270 × 120–140 µm; swollen agglutinated hairs forming a collaret at the base of the neck and on upper peridium 1-3 cells, hyaline to pale brown with darker apices 12–30 × 5–10 µm, terminal cell of each hair more elongate; ostiole 60–110 µm diam. *Peridium* membranaceous, semi-transparent; *endoperidium* pseudoparenchymatous of thin walled cells; *mesoperidium* of flattened parallel cells; *exoperidium* a *textura globulosa-angularis* with cells 11–13 × 6.5–11 µm from which hyphoid hyaline to pale brown hairs originate. *Jacket paraphyses* ephemeral, hyaline, 8–10.5 × 6–7 µm broad, septate, surrounding the asci, with hyaline vacuoles. *Asci* 32-spored, 200–260 × 38–52 µm, clavate-lageniform, swelling in water, slightly pointed apex, indistinct apical ring, contracted below into a stipe 40–80 µm × 5–6.5 µm. *Ascospores* irregularly multi-seriate, two-celled at maturity; dark cell 19–23 × 11.5–14.5 µm, light greenish to olivaceous brown in reflected light, ellipsoidal, smooth, flattened at the base, with slightly umbonate apices and an apical germ pore, ca. 1.5 µm diam.; pedicel 6–7.5 × 1.5–2 µm, cylindrical, plasma filled, persistent; lash-like *caudae* one from apex of spore head, hollow, eccentric to germ pore and the other surrounding the whole pedicel, 25–36 µm long.



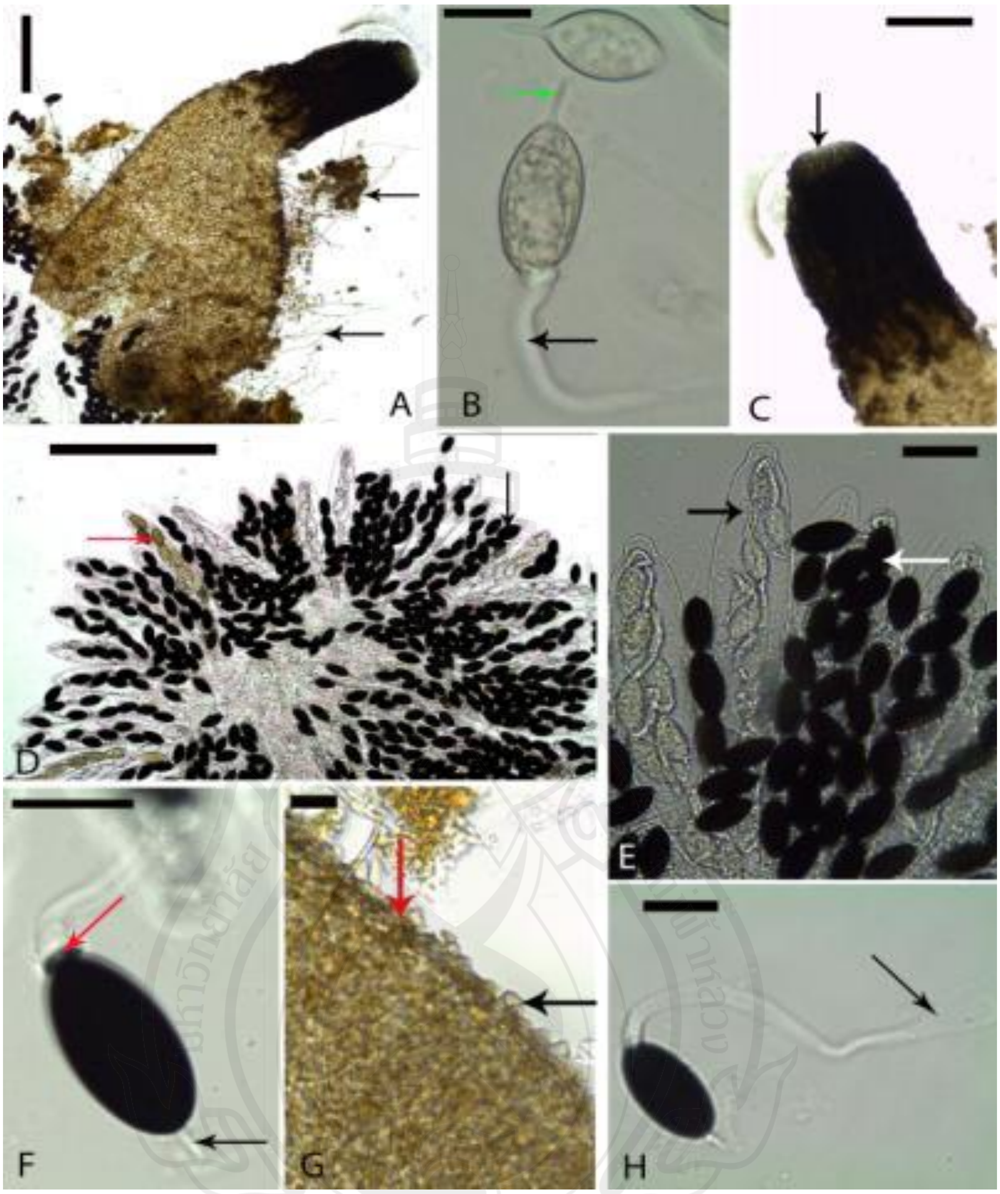
Note. (A) Superficial ascomata on dung, note hairs on necks (arrows). (B) Squashed ascoma. (C) Mature and immature asci with ascospores. (D) Perithecial neck. (E) Ascospores showing pedicels (arrows). (F) Agglutinated hairs at the neck. (G) Ascus stipe (arrow) and free ascospores. (H) Jacket paraphyses, note septation (arrows). (I) Exoperidial wall. Scale bars: (A) = 500 µm, (B) = 200 µm, (C-D) = 50 µm, (E-I) = 20 µm.

Figure 6.3 *Schizothecium dakotense* (KWSNNP010-2009).

Schizothecium dakotense is close to *S. alloeochaetum* (Mirza & Cain) L. Cai from which it is differentiated by having somewhat larger ascospores {spore size in *S. alloeochaetum* = 15.5–18.5 × 11–13.5 (Mirza & Cain, 1969)}. *Schizothecium formosanum* (Y-Z Wang) L. Cai is differentiated from *S. dakotense* by the 64-spored asci and lower cauda not covering the whole pedicel. *Schizothecium simile* (E.C. Hansen) N. Lundq. has both 16 and 32-spored asci but can be differentiated by its larger spores with a sheath and the presence of rigid hairs at the perithecial neck. The morphological features of our Kenyan collections of *Schizothecium dakotense* match those of the Japanese, Italian and Australian collections (Furuya & Udagawa, 1972; Bell, 2005).

6.3.4 *Schizothecium dubium* (E.C. Hansen) N. Lundq Symb. Bot. Upsal. 20 (1): 254, 1972. (Figure 6.4 A-H)

Ascomata perithecioid, superficial, 650–1030µm high, 300–500 µm diam., scattered, subglobose to pyriform, exposed part covered with long flexuous septate, olivaceous-brown hairs 2–3.5 µm broad. *Neck* 255–330 × 160–175 µm, black, usually straight, cylindrical, with fascicles of agglutinated short brown hairs, consisting of swollen cells. Some swollen hairs also present in the upper part of the venter. *Peridium* membranaceous, semi-transparent; endostratum *textura angularis* of small polygonal cells and exostratum *textura globulosa angularis* of roundish to polygonal cells with roundish. *Jacket paraphyses* moniliform surrounding the asci, hyaline, septate, exceeding the asci. *Asci* 16-spored, 252–302 × 33–56 µm, unitunicate, fusiform-clavate, broadly rounded above, tapering below into a long, thin crooked stipe, with an indistinct apical ring. *Ascospores*, two-celled at maturity, biseriate; apical dark cell 34–40 × 16.5–20 µm, ellipsoidal, hyaline at first, through olivaceous to dark brown and opaque, with a wide apical germ pore; *pedicel* slender, sub-cylindrical to obconical, 6–7.5 × 2.0–2.5 µm, persistent; *caudae* lash-like, granulose, upper one hollow, 65–100 × 4–10 µm, the end broader than the base; lower one hyaline, enveloping the pedicel, ca. 50 × 5.5 µm.



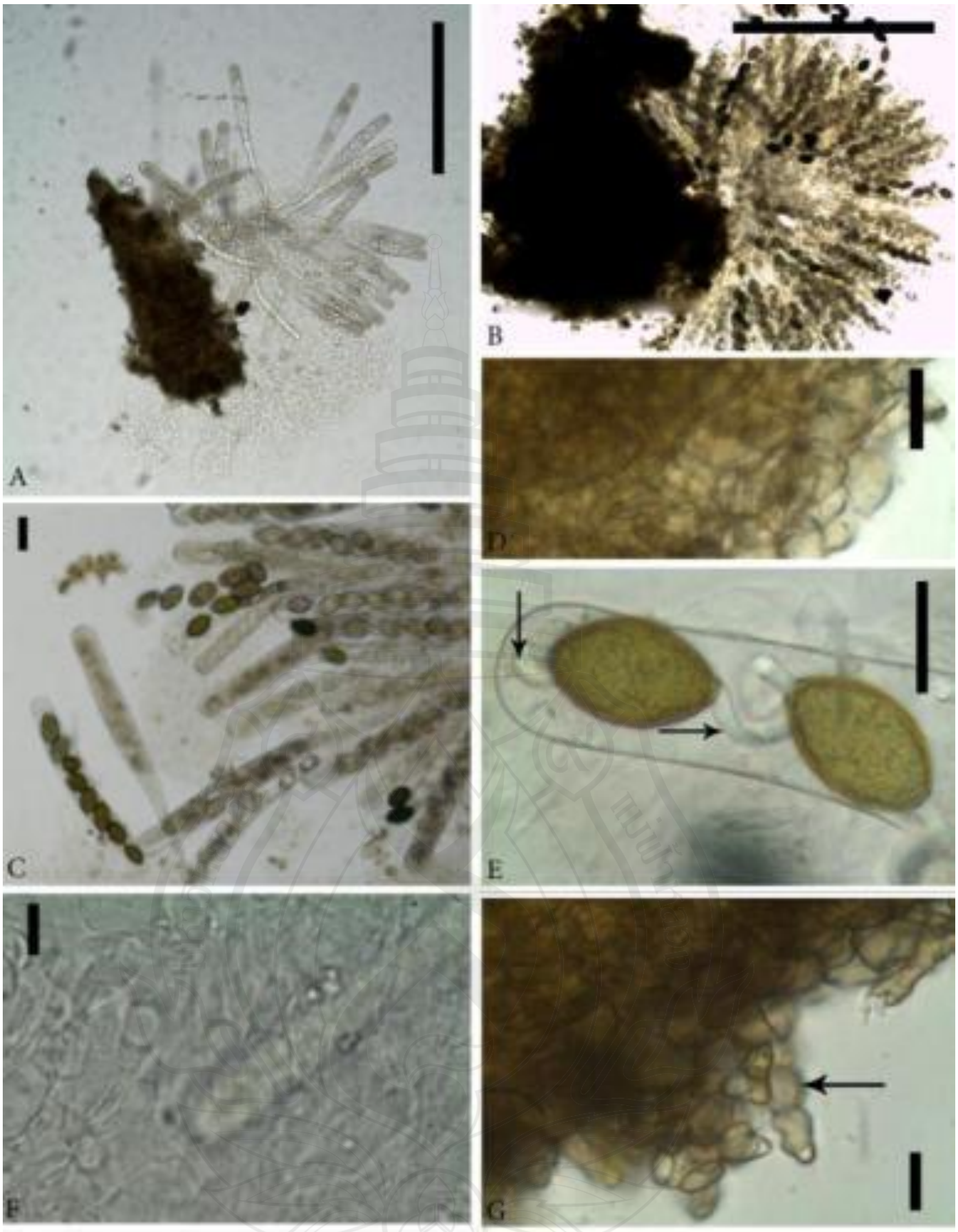
Note. (A) Squashed perithecium showing long flexuous hairs (arrows). (B) Free immature spores, note cauda (black arrow) and pedicel (green arrow). (C) Neck showing ostiole (arrow). (D) Section of centrum showing mature asci with ascospores. (E) Mature (white arrow) and immature asci (black arrow). (F) Free mature ascospore, note pedicel (black arrow) and upper cauda over a wide germ pore (red arrow). (G) Details of peridium, note swollen hairs (red and black arrows). (H) Free mature ascospore showing lash-like cauda with broadened end (arrow). Scale bars: (A, C-D) = 200 µm, (E, G) = 50 µm, (B, F, H) = 20 µm.

Figure 6.4 *Schizothecium dubium* (KWSNNP008-2009).

Schizothecium dubium has some similarities with the 16-spored form of *S. simile* (E.C. Hansen) N. Lundq. from which it is distinguished by lacking rigid, erect hairs on the neck, lacking a gelatinous sheath around the spore head, and having a broader hollow rather than solid upper cauda (Cain, 1934; Lundqvist, 1972; Doveri, 2008). *Schizothecium dubium* is also similar to *S. dakotense* but the latter not only has smaller 32-spored asci but also smaller ascospores and perithecia (Lundqvist, 1972; Doveri, 2008). *Schizothecium dubium* is a new record for Kenya.

6.3.5 *Schizothecium glutinans* (Cain) N. Lundq., Symb. Bot. Upsal. 20 (1): 254, 1972. (Figure 6.5 A-G)

Ascomata perithecioid, immersed with only the neck protruding, rarely semi-immersed, 450–610 × 165–340 µm, scattered, broadly ellipsoidal to pyriform, membranaceous, semi-transparent, olivaceous brown, with a papilliform, bluntly conical, black neck covered at its base with a crown of short 10–25 µm long articulated (one to three cells high), swollen hairs forming almost triangular scales. Some scattered swollen hairs also present on the remaining part of the perithecium. Lower part of the perithecium also with short, flexuous, densely septate hairs. *Peridium* 2-layered, pseudoparenchymatous. *Endoperidium* of pale, thin-walled polygonal cells. *Exoperidium* a *textura globulosa-angularis* of thick-walled cells 4–8 × 5–10 µm. *Jacket paraphyses* hyaline, reduced to shapeless material surrounding the asci, vanishing. *Asci* 8-spored, 250–336 × 25–32 µm, cylindrical, apex slightly rounded, apical ring indistinct, long stipitate. *Ascospores* two-celled at maturity, usually obliquely uniseriate, rarely biseriate; apical dark cell 26.5–30.5 × 15.5–20 µm, olivaceous-black, broadly ellipsoidal, with a small germ pore at the slightly umbonate apex; pedicel hyaline, straight or slightly curved, cylindrical, 6.5–13.5 × 2–3.5 µm, persistent; *cauda* at apex of pedicel long and lash-like, longer than the apical one, 4–4.5 µm diam., continuous with a thin gelatinous sheath over the pedicel; the other *cauda* at apex of spore, covering the germ pore, broader , lash-like, furrowed, 3–7.5 µm diam.



Note. (A-B) Ascoma squash. (C) Immature asci and ascospores. (D) Details of peridium. (E) Immature ascospores in ascus apex, note gelatinous appendages (arrows). (F) Paraphyses. (G) Agglutinated hairs and swollen cells (arrow). Scale bars: (A-C) = 200 µm, (D-G) = 20 µm.

Figure 6.5 *Schizothecium glutinans* (KWSSH005A-2008).

Schizothecium glutinans is closely related to *S. miniglutinans* (J.H. Mirza & Cain) N. Lundq, however the former has larger asci and ascospores (Bell & Mahoney, 1995; Doveri, 2008). Ascospore size range for this Kenyan collection (26.5–30.5×15.5–20 μm) is very close to those reported by a majority of previous investigators (Cain, 1934; Mirza & Cain, 1969; Lundqvist, 1972; Bell & Mahoney, 1995; Doveri, 2004, 2008; Bell, 2005). This species is a new record for Kenya.

6.4 Conclusions

Dung from Cape buffalo, zebra, giraffe, hippopotamus, impala, Jackson's hartebeest, sable antelope and waterbuck was incubated and screened for coprophilous *Schizothecium* species. Using morphological features, six taxa were identified. The species included *Schizothecium aloides*, *S. conicum*, *S. curvuloides* var. *curvuloides*, *S. dakotense*, *S. dubium* and *S. glutinans*. *Schizothecium dakotense*, *S. dubium* and *S. glutinans* were new records. *Schizothecium curvuloides* var. *curvuloides* and *S. dakotense* were fairly common.

CHAPTER 7

COPROPHILOUS ASCOMYCETES: V. Sordariales Chadef. ex D. Hawksw. & O.E. Erickss.

7.1 Introduction

The Order Sordariales comprises saprobic fungi with ascomata that are usually perithecioid or occasionally cleistothecioid growing on dung or decaying plant biomass, and are membranaceous to coriaceous and glabrous or hairy (Lundqvist, 1972; Huhndorf et al., 2004; Doveri, 2004, 2008). Asci are thin-walled, unitunicate or proto-tunicate, sometimes with non-amyloid apical structures or may lack an apical apparatus, and usually sandwiched between paraphyses (Lundqvist, 1972; Doveri, 2004, 2008; Bell, 2005). Ascospores are hyaline to dark, one- to poly-celled, with germ pore(s) or slit(s) and often with gelatinous appendages or sheaths (Lundqvist, 1972; Huhndorf et al., 2004; Bell, 2005).

The aim of this survey is to document the taxonomy, diversity, occurrence and distribution of Sordariales from wildlife dung that did not sporulate in sufficient number of taxa to be described each on its own as a single genus. Species from the genera *Arnium* Nitschke ex G. Winter emend. N. Lundq., *Zopfiella* G. Winter, *Zygopleurage* Boedijn and *Sordaria* Ces. & De Not. are described and illustrated.

7.2 Materials and Methods

Dung samples from eight wild animal species namely giraffe, Kirk’s dikdik, African elephant, buffalo, zebra, waterbuck, impala and Jackson’s hartebeest were collected and incubated for observing the presence of coprophilous ascomycetes as described in Section 2.2 of Chapter 2.

The Sordariales species described below were classified based on morphological characters as described in monographs and dichotomous keys on members of the Order Sordariales (Cain, 1934; Lundqvist, 1972; Bell, 1983, 2005; Richardson & Watling 1997; Doveri, 2004).

7.3 Results and discussion\

Arnium Nitschke ex G. Winter species have non-stromatic, and perithecioid ascomata covered with various kinds of hairs (Lundqvist, 1972). They are paraphysate, rarely aparaphysate and their asci are 4- to multi-spored, usually cylindrical to clavate with or without an apical apparatus (Lundqvist, 1972). Ascospores may be uniseriate, biseriate or multiseriate, one-celled and sometimes two-celled as a result of a transverse septum developing after pigment formation, brownish black, smooth, ellipsoidal to broadly fusiform, with 1 or 2 germ pores and usually one gelatinous cauda at each end (Lundqvist, 1972). This genus is very close to Podospora Ces. but is easily distinguished by having ascospores lacking a pedicel in addition to the characteristic ellipsoidal or fusiform immature ascospores. Arnium is usually cosmopolitan and coprophilous and grows on various herbivore dung types (Lundqvist, 1972; Bell, 2005).

Zopfiella G. Winter species usually have dark to olivaceous brown, non-stromatic, usually superficial cleistothecia with a peridium adorned with varying degrees of hair. They have highly evanescent asci that usually are 8-spored, cylindrical to clavate, lacking an apical apparatus (Udagawa & Furuya, 1974; Huhndorf et al., 2004; Bell, 2005; Cai et al., 2006; Kirk et al., 2008). *Ascospores* lack a gelatinous equipment, are 1-celled and hyaline in the early stages, transversely septate and 2- sometimes 3-celled at maturity, with an ellipsoidal, smooth, pigmented, often dark brown apical cell, and a hyaline, basal pedicel. The apical cell (head) has an apical or subapical germ pore (Udagawa & Furuya, 1974; Guarro et al., 1991; Huhndorf et al., 2004; Bell, 2005; Kirk et al., 2008). This genus is cosmopolitan (Guarro et al., 1991) and has been isolated from dung and soil. *Zopfiella* and

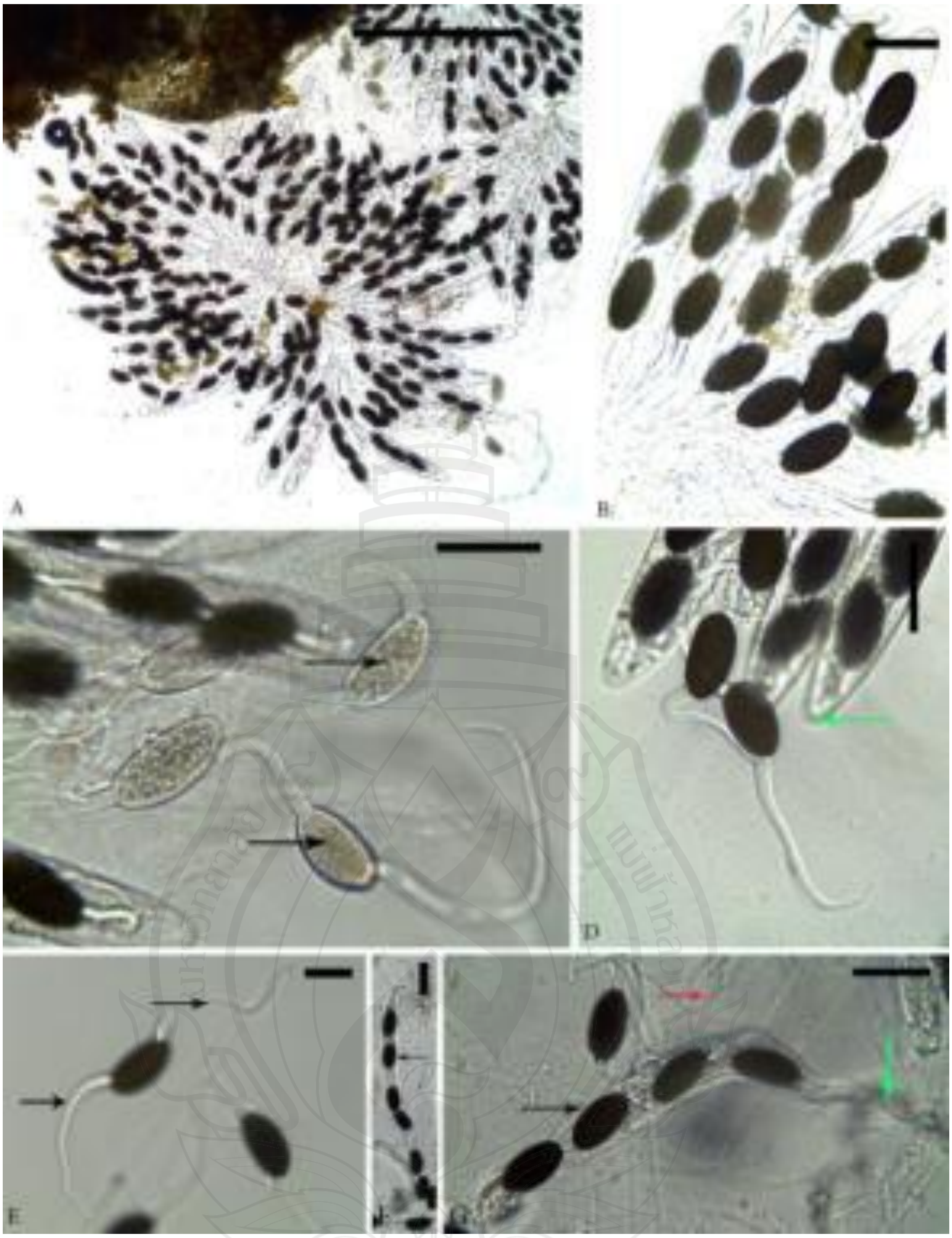
Podospora are similar but the former can be distinguished by its cleistothecial ascomata and ascospores without gelatinous equipment and with short, easily collapsing pedicels. The pedicel shape is a very important character in delimiting species.

Zygopleurage Boedijn is distinguished from other Lasiosphaeraceae by its unique ascospore morphology which consists of two dark ellipsoidal cells connected by an elongated cylindrical hyaline cell. The hyaline, intercalary cells are often coiled in the central part of ascus before spore discharge and separate two sets of polar pigmented cells. The size and shape of the intercalary cells and pigmented cells (spore heads), with their gelatinous sheath, and claw-shaped appendages vary in *Zygopleurage* and are very useful in species delimitation. Currently there are only three described species of *Zygopleurage*: *Z. faiyumensis* N. Lundq., *Z. multicaudata* Mirza and *Z. zygospora* (Speg.) Boedijn. This unique coprophilous and cosmopolitan genus was reported by Lundqvist (1969) from Europe, North America, South America and Africa. Records from other parts of the world include Thailand in South East Asia (Mungai et al., 2011), South America (Richardson, 2001), the Middle East (Abdullah & Rattan, 1978) and Australia (Bell, 2005).

Sordaria Ces. & De Not. is characterized by dark, superficial or semi-immersed, non-stromatic perithecia and a layered, pseudoparenchymatous peridium (Cain, 1934; Lundqvist, 1972; Bell, 2005). Asci are unitunicate, non-amyloid, cylindrical, usually 8-spored, each with a well developed apical apparatus (Lundqvist, 1972; Bell, 2005). Ascospores are one-celled, broadly ovoid to ellipsoidal, sometime subglobose or subfusiform, dark pigmented at maturity, with a basal germ pore and usually surrounded by a hyaline mucilaginous sheath (Lundqvist, 1972; Bell, 2005). *Sordaria* species have very similar morphological features thus creating a challenge in species delimitation. According to Lundqvist (1972) and Guarro & von Arx (1987) analysis of the perithecial structure, ascus and spore size is a very reliable way of delimiting *Sordaria* species. This genus is composed of mainly fimicolous species. *Sordaria* has been recorded worldwide (Cailleux, 1971; Lundqvist, 1972; Khan & Krug, 1989a; Bell, 2005; Jeamjitt et al., 2007; Richardson, 2008). The detailed descriptions for each species are as provided in the sections below.

7.3.1 *Arnium arizonense* (Griffiths) N. Lundq. & J.C. Krug, Symb. Bot. Upsal. 20 (no.1): 232, 1972. (Figure 7.1 A-F)

Ascomata perithecioid, semi-immersed to superficial, 300–600 µm diam., scattered or gregarious, membranaceous to slightly coriaceous, black to translucent, pyriform. *Neck*, black, opaque sometimes curved, papilliform to cylindrical, with long, one-sided tufts of rigid and septate hairs. *Peridium* pseudoparenchymatous, layered, olivaceous brown. *Paraphyses* numerous, simple, broadly filiform, septate, exceeding the asci, variable width and tapering. *Asci* 4-spored, 251–350 × 30–39 µm, unitunicate, clavate, with a thickened apical ring, wall non-amyloid, with a slightly pointed apex, apical membrane thickened, sub-apical chamber 5–7 µm broad long stipitate; stipe crooked, 100–150 × 25–35 µm. *Ascospores* 43.5–48.5 × 22–29 µm, obliquely uniseriate, one-celled, ellipsoidal, sometimes inequilateral, initially hyaline, later changing through yellowish, olivaceous to brown-black, smooth, thick walled, tipped at each end with a long gelatinous cauda, almost equal in length and same morphology, lash-like, 8–10 µm broad at base and over 100 µm long, persistent, not swelling in water, solid, often densely transversely segmented, occasionally faintly longitudinally and proximally furrowed; one cauda sub-apical, on the flattened side of the spore, not covering the germ pore; the other cauda somewhat eccentric on the same direction of opposite side of spore. *Germ pore* apical.



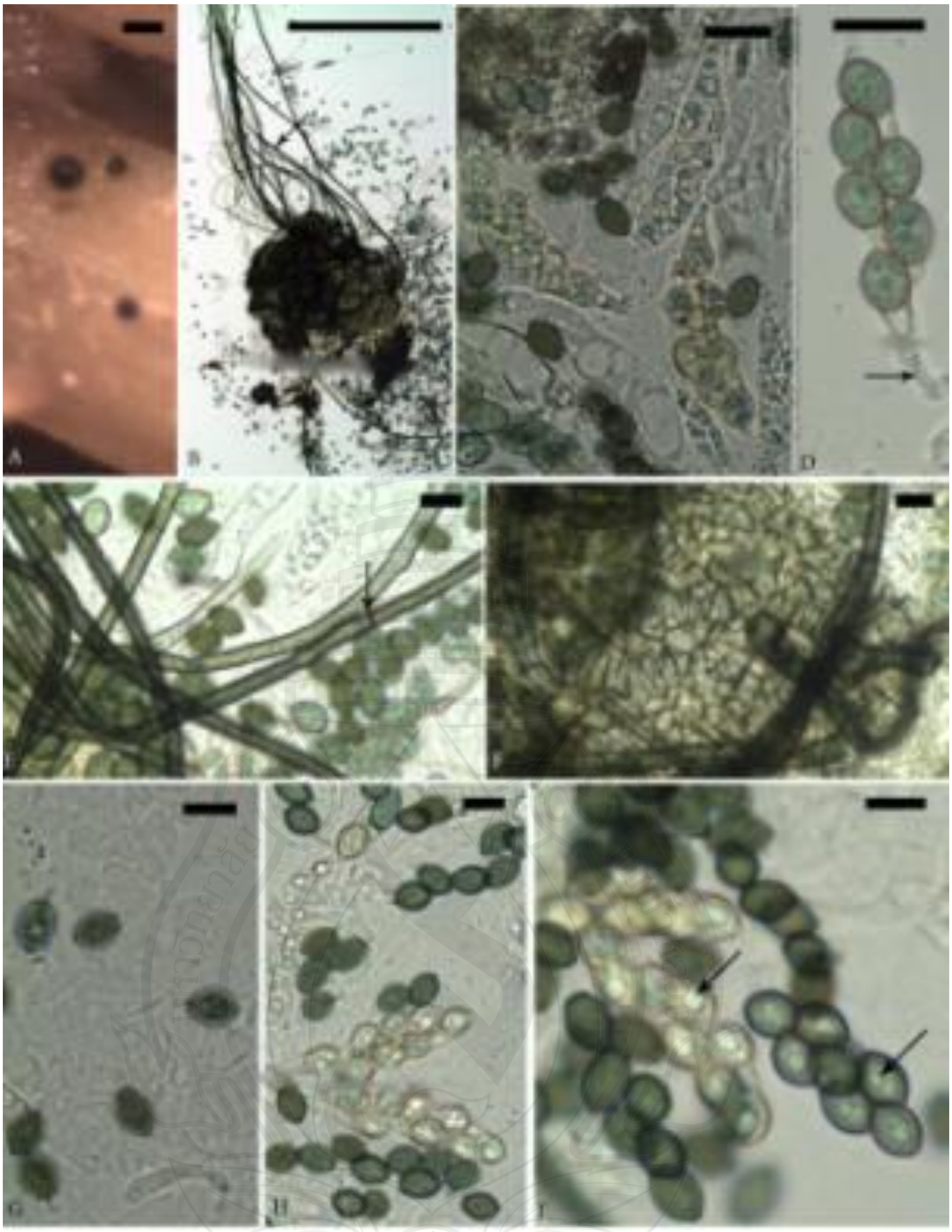
Note. (A-B) Asci and ascospores. (C) Immature inequilateral ascospores (arrows). (D) Apical chamber of asci with spores and free mature ascospores, note apex (green arrow). (E) Free mature ascospores showing almost equal sized caudae (arrows). (F-G) Ascus showing uniseriate spore arrangement (black arrows), paraphyses (red arrow) and stipe (green arrow). Scale bars: (A, F) = 200 µm, (B, C, D, G) = 50 µm.

Figure 7.1 *Arnium arizonense* (KWSNNP017B-2010).

Arnium arizonense is similar to *Podospora australis* (Speg.) Niessl, but *P. australis* has a prominent apiculum on each narrowly ovoid spore and does not have rigid, agglutinated neck hairs (Lundqvist, 1972). *Arnium hirtum* (E.C. Hansen) N. Lundq. & J.C. Krug is another similar taxon having sometimes 4-spored asci, but it has non-fasciculate neck hairs (Bell, 2005) and differently placed and structured gelatinous caudae. *Arnium arizonense* is a new record for Kenya.

7.3.2 *Zopfiella* affinis *erostrata* (Griffiths) Udagawa & Furuya, Trans. Mycol. Soc. Japan 15: 208, 1974. (Figure 7.2 A-I)

Ascomata cleistothecioid, superficial, 280–300 µm diam., scattered or in small groups, black, globose to subglobose, with long flexuous, olivaceous brown to dark, robust and septate hairs evenly distributed, 2–5 µm broad, hair ends straight, smooth and pointed; *Peridial wall* olivaceous brown, membranaceous, *textura angularis* of cells 5.5–8 × 4.5–6 µm. *Paraphyses* not observed. *Asci* 8-spored, 43–56 × 10–14.5 µm, unitunicate, clavate, broadly rounded above and tapering below into 8–15 µm long, very evanescent stipes, apical apparatus not distinct/observed, surrounded by hyaline swollen cells, collapsing in water mounts. *Ascospores* irregularly biseriate, hyaline at first and one-celled, transversely septate later and two-celled. Upper cell 9.5–11 × 6.5–8 µm, broadly limoniform, umbonate, with truncate base, grayish to black, guttulate; *pedicel* 4–6 × 1.5–4.5 µm, triangular, soon collapsing. *Germ pore* apical.



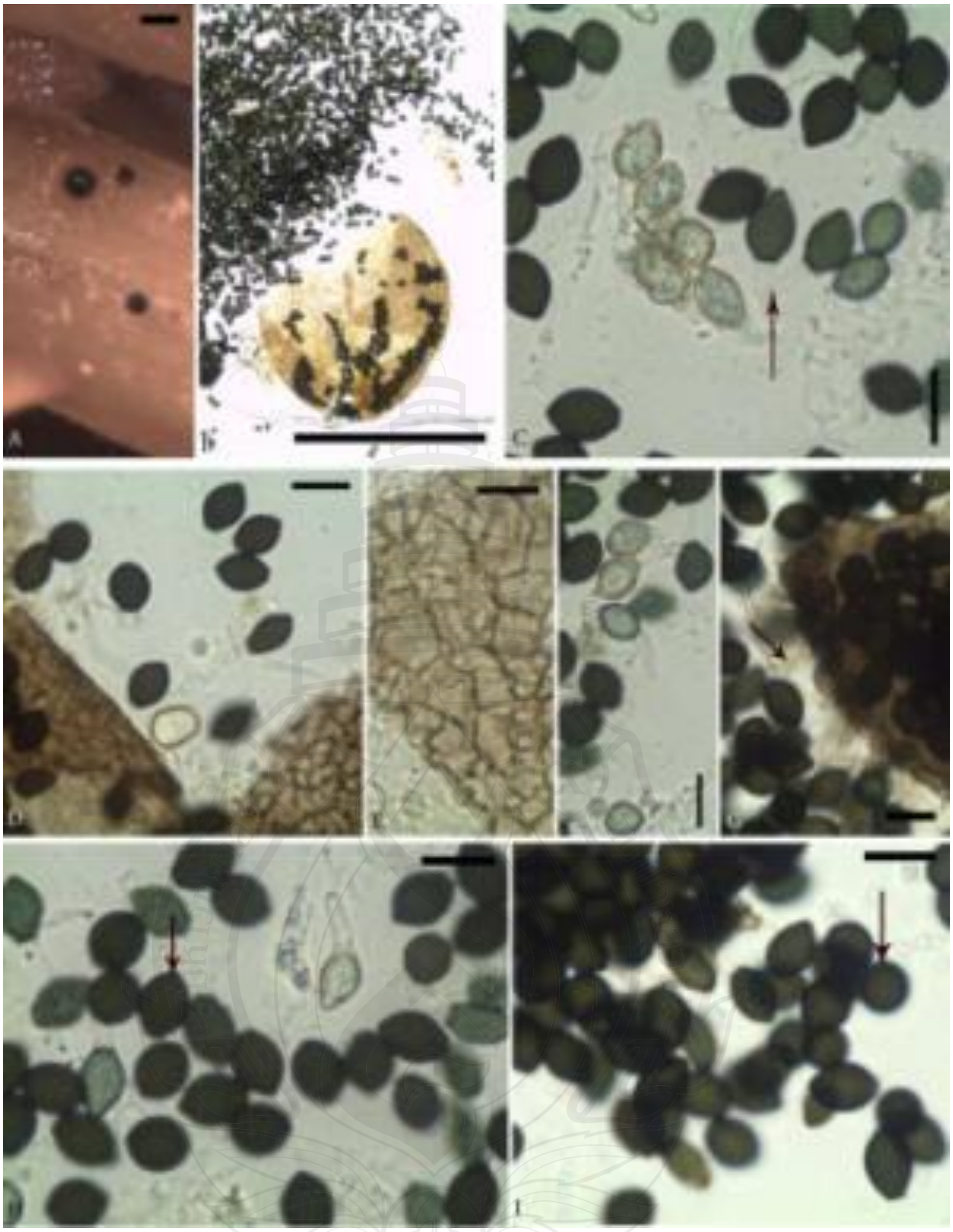
Note. A Cleistothecia on dung. B Squashed cleistothecium showing long flexuous hairs. C Asci and ascospores at various stages of maturity. D Ascus, note stipe (arrow) E Cleistothecial hairs, note septation (arrow). F Peridial wall. G Free ascospores amongst hyaline swollen cells. H Free mature ascospores amongst immature asci. I Asci and ascospores showing oil droplets (arrows). Scale bars: A = 500 µm, B = 200 µm, C-I = 20 µm.

Figure 7.2 *Zopfiella* affinis *erostrata* (KWSTE005B-2009).

Apart from the triangular pedicel *Zopfiella* affinis *erostrata* has matching features to descriptions of *Zopfiella erostrata* from Australia and Japan (Udagawa & Furuya, 1974; Bell, 2005). It is also close to *Zopfiella longicaudata* but the latter has larger spore heads and pedicels and sporulates late in incubation. This specimen does not fit the existing keys on account of the triangular and small ascospores.

7.3.3 *Zopfiella longicaudata* (Cain) Arx, Proc. Konik. Nederl. Akad. van Wetensch. 76(3): 291, 1973. (Figure 7.3 A-I)

Ascomata cleistothecioid, superficial, 170–220 µm diam., scattered or in small groups, brown, globose to subglobose, with olivaceous brown to light grey, septate, unbranched hairs evenly distributed; hairs 10.5–18 × 2–3 µm, hair ends almost straight, smooth and blunt. *Peridial wall* semi-transparent, olivaceous brown, *textura angularis* of polygonal cells 7–11.5 × 4.5–10 µm. *Paraphyses* not observed. *Asci* 8-spored, 61–82 × 13.5–16 µm, unitunicate, clavate to cylindrical, broadly rounded above and tapering below into evanescent long stipes measuring 10–17 × 3–4 µm, lacking apical apparatus, surrounded by hyaline swollen cells, collapsing in water mounts. *Ascospores* irregularly biseriate, hyaline at first and one-celled, transversely septate later and two-celled. Upper cell 13–17.5 × 9.5–11 µm, broadly limoniform, slightly inequilateral, initially light grayish to black, with a truncate base; *pedicel* 8–11 × 3–4 µm, hyaline, slightly curved, cylindrical with rounded ends, collapsing with maturity, umbonate at the apex, immature ascospores guttulate. *Germ pore* conspicuous, sub-apical.



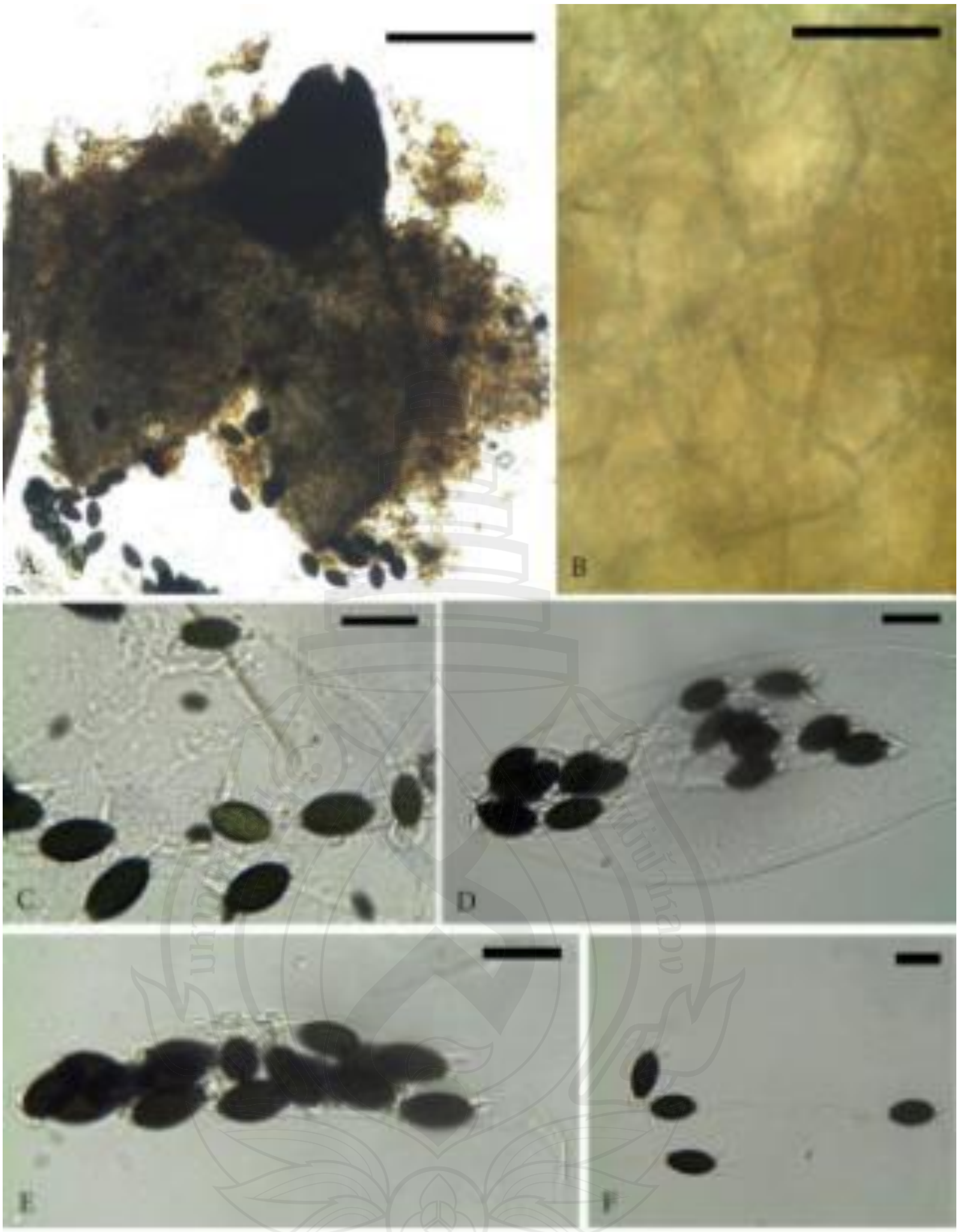
Note. (A) Ascomata on dung. (B) Squashed ascoma. (C) Immature ascus among free mature ascospores, note pedicels (arrow). (D-E) Details of peridial wall. F Immature and mature ascospores. (G) Ascomal wall section showing hairs (arrow). (H-I) Ascospores, note germ pores (arrows). Scale bars: (A) = 500 µm, (B) = 200 µm, (C-I) = 20 µm.

Figure 7.3 *Zopfiella longicaudata* (KWSTE005B-2009).

The ascospores of *Zopfiella longicaudata* measuring 9.5–11.5 × 6.5–8 µm (in this study) are larger than those of *Z. erostrata* but smaller than *Z. flammifera* L.H. Huang, which measure 16–21.5 × 9.5–13 µm (Doveri, 2004). *Zopfiella longicaudata* is apparently more frequent than *Zopfiella* affinis *erostrata* and sporulates very late on incubated wildlife dung. *Zopfiella longicaudata* is a new record for Kenya.

7.3.4 ***Zygopleurage zygospora*** (Speg.) Boedijn, Persoonia 2: 316, 1962. (Figure 7.4 A-F)

Ascomata perithecioid, immersed to semi-immersed, 600–1340 µm high, 400–760 µm diam., scattered or in small groups, olivaceous brown, pyriform, with a venter usually covered with long, brown, septate, flexuous hairs. *Neck* 200–530 × 120–370 µm, cylindrical, covered with short hair-like cells, darker, ostiole 105–115 µm diam. *Peridium* 3-layered; *exoperidium* thin, semi-translucent of *textura angularis* cells, 65 µm thick, *mesoperidium* of smaller vertically elongated cells, *endoperidium* consisting of subhyaline to light brown *textura angularis* cells. *Paraphyses* simple, hyaline, septate, evanescent. *Asci* 8-spored, 250–322 × 40–49.5 µm, clavate, unitunicate, long-stipitate, rounded apex. *Ascospores* filamentous, one-celled and hyaline when young, loosely coiled in the ascus, 3-celled at maturity, composed of two dark brown end cells, 29–37 × 17.5–23 µm, usually smooth, ellipsoidal, each with an apical germ pore, joined by a long subhyaline intercalary cell, cylindrical, 211– 228 × 5.5–7.5 µm; 7–9 µm broad at the point of insertion to dark cell, staining blue in lactophenol cotton blue, usually parallel or coiled, each dark end cell with 4 distinct, short, claw-shaped, hyaline, apical, gelatinous appendages, 11–15 × 3–4 µm long and 4 short gelatinous caudae arising at the septa of the intercalary cell, 10–13 × 3–4 µm.



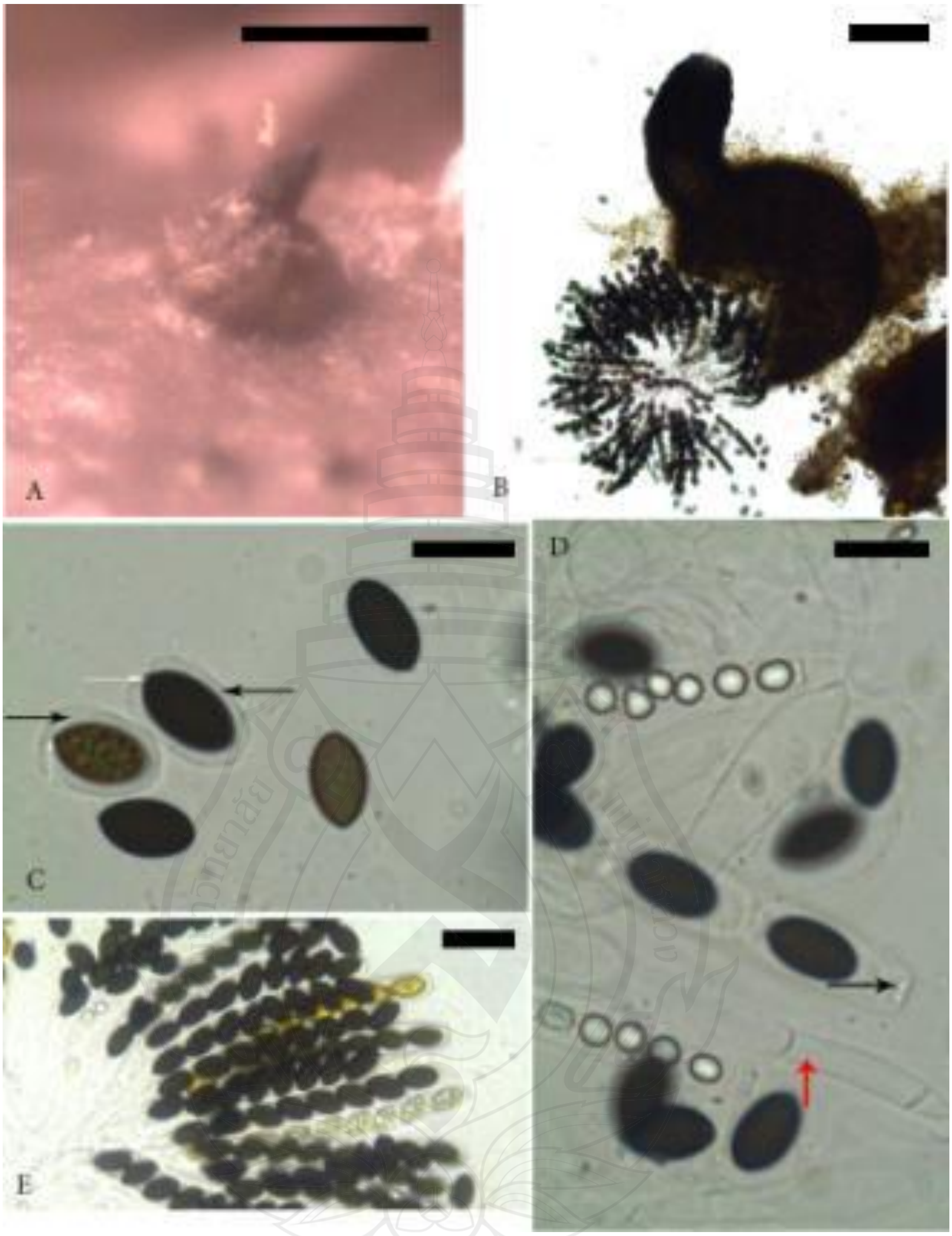
Note. (A) Squashed ascoma. (B) Details of peridial wall. (C) Free mature ascospores. (D) Mature asci with ascospores showing spore arrangement, intercalary cell and short caudae. (E) Mature ascus with ascospores. (F) Free mature ascospores. Scale bars: (A) = 500 µm, (B) = 20 µm, (C-F) = 50 µm.

Figure 7.4 *Zygopleurage zygospora* (KWSNNP002-2009).

Zygopleurage zygospora is characterized by ascospores with longer intercalary cells always coiled inside the asci and four distinct, short, claw-shaped, hyaline gelatinous processes on the polar cells (Abdullah & Rattan, 1978). These characters vary within the taxa of *Zygopleurage* and are very useful in delimitation (Lundqvist, 1969, 1972; Abdullah & Rattan, 1978). In addition, *Z. zygospora* has an olivaceous brown perithecium. The ascospores of *Z. zygospora* are intermediate in size between those of *Z. multicaudata*, which are smaller and *Z. faiyumensis*, which are larger (Abdullah & Rattan, 1978). *Zygopleurage zygospora* is a very unique species and therefore not easy to confuse with other species (Lundqvist, 1969, 1972; Abdullah & Rattan, 1978).

7.3.5 *Sordaria fimicola* (Roberge) Ces. & De Not., Comm. Soc. Critt. Ital. 1: 226, 1863. (Figure 7.5 A-E)

Ascomata perithecioid, semi-immersed to superficial, 550–620 µm, high, 450–500 µm, diam., scattered or more often gregarious or even crowded, membranaceous, dark brown, sparsely covered with hyphoid hairs, ovoid to pyriform. *Neck* conical or subcylindrical, 100–120 × 120–150 µm. *Peridial wall* layered, pseudoparenchymatous; exostratum a *textura angularis* of polygonal cells in the venter, a *textura globulosa* in the neck, 10.5–17 × 8.5–13 µm. *Paraphyses* moniliform, septate, with segments 4.5–12.5 µm broad, abundant, containing hyaline vacuoles. *Asci* 8-spored, 111–163 × 10.5–14 µm, cylindrical, flattened at apex, short stipitate, with a lobate stipe, and prominent apical apparatus. *Ascospores* 15.5–18.5 × 9.5–11.5 µm, obliquely to vertically uniseriate, dark brown, ellipsoidal, occasionally ovoid, smooth, slightly pointed and apiculate at the base, surrounded by a gelatinous sheath usually invaginated at the apiculum. *Germ pore* single and basal.



Note. (A) Ascoma on dung. (B) squashed ascoma. (C) Free mature ascospores showing gelatinous sheath and germ pore (arrows). (D) Asci apex and paraphyses. (E) Mature asci with ascospores. Scale bars: (A) = 500 µm, (B) = 200 µm, (C-D) = 20 µm, (E) = 50 µm.

Figure 7.5 *Sordaria fimicola* (KWSKIN004-2009).

Sordaria species are very homogenous and therefore are very difficult to delimit. *Sordaria fimicola* differs from *S. superba* De Not. and *S. macrospora* Auersw. by having smaller ascospores, ellipsoidal rather than broadly ellipsoidal and smaller perithecia and asci (Doveri, 2004). Other similar taxa namely *S. sibutii* Cailleux and *S. conoidea* Cailleux lack a gelatinous perisporium on their spores. *S. fimicola* is homothallic with four hardly differentiated heterothallic relatives, namely, *S. thermophila* Fields, *S. sclerogenia* Fields & Grear, *S. tomentoalba* Cailleux and *S. brevicollis* L.S. Olive & Fantini (Doveri, 2004). *Sordaria fimicola* is reportedly a very common cosmopolitan pyrenomycete however, it was isolated only once in this study (Lundqvist, 1972; Doveri, 2004). This is a new record for Kenya.

7.4 Conclusions

Dung collected from African elephant, Cape buffalo, dikdik, giraffe, impala, Jackson's hartebeest, waterbuck and zebra was incubated to culture coprophilous Sordariales. Upon examination, *Arnium arizonense*, *Zopfiella* affinis *erostrata, Z. longicaudata*, *Sordaria fimicola* and *Zygopleurage zygospora* were described and illustrated.

Arnium arizonense, *Sordaria fimicola* and *Zopfiella longicaudata* are reported for the first time in Kenya. *Zygopleurage zygospora* is a very frequent species on wildlife dung.

CHAPTER 8

COPROPHILOUS ASCOMYCETES: VI. *Sporormiella* Ellis & Everh.

8.1 Introduction

Sporormiella in the family Sporormiaceae Munk is the most species-rich in Sporormiaceae (Barr, 2000). *Sporormiella* has been recorded worldwide for instance, from Africa (Ebersohn & Eicker, 1992; Caretta et al., 1998), Europe (Richardson, 1998; Doveri, 2004; Kruys, 2005), Asia (Furuya & Udagawa, 1972; Piasai et al., 2009; Mungai et al., 2011) and the Americas (Angel & Wicklow, 1983; Richardson, 2008). This genus is closely related to *Preussia* Fuckel and *Sporormia* De Not.

Records of *Sporormiella* from Kenya include *Sporomiella minima* (Auersw.) Ahmed & Cain, *S. australis* (Speg.) Ahmed & Cain, *S. capybarae* (Speg.) Ahmed & Cain, *S. dubia* Ahmed & Cain, *S. herculea* (Ell. & Ev.) Ahmed & Cain, *S. intermedia* (Auersw.) Ahmed & Cain, *S. isomera* Ahmed & Cain, *S. kansensis* (Griff.) Ahmed & Cain, *S. longispora* (Cain) Ahmed & Cain, *S. longisporopsis* Ahmed & Cain, *S. macropulchella* Khan & Cain, *S. megalospora* (Auersw.) Ahmed & Cain, *S. minima* (Auersw.) Ahmed & Cain, *S. minimoides* Ahmed & Cain, *S. obliqua* Khan & Cain *S. muskokensis* Ahmed & Cain, *S. pilosa* (Cain) Ahmed & Cain, *S. similis* Khan & Cain, *S. subtilis* Ahmed & Cain, *S. tenuispora* Khan & Cain, *S. teretispora* Ahmed & Cain, *S. tetramera* Ahmed & Cain (Minoura, 1969; Khan & Cain, 1979; Caretta et al., 1998).

The objectives of this survey were to describe *Sporormiella* from wild herbivore dung in Kenya and to document species diversity and distribution in relation to different wild herbivore dung types.

8.2 Materials and Methods

Dung collected from African elephant, Cape buffalo, dikdik, giraffe, impala, zebra, and waterbuck was incubated for the study of coprophilous ascomycetes as described in Section 2.2 of Chapter 2.

Sporormiella species described in this work were identified based on morphological characters as described in monographs and dichotomous keys on members of the genus (Cain, 1934; Lundqvist, 1972; Bell, 1983, 2005; Richardson & Watling, 1997; Doveri, 2004).

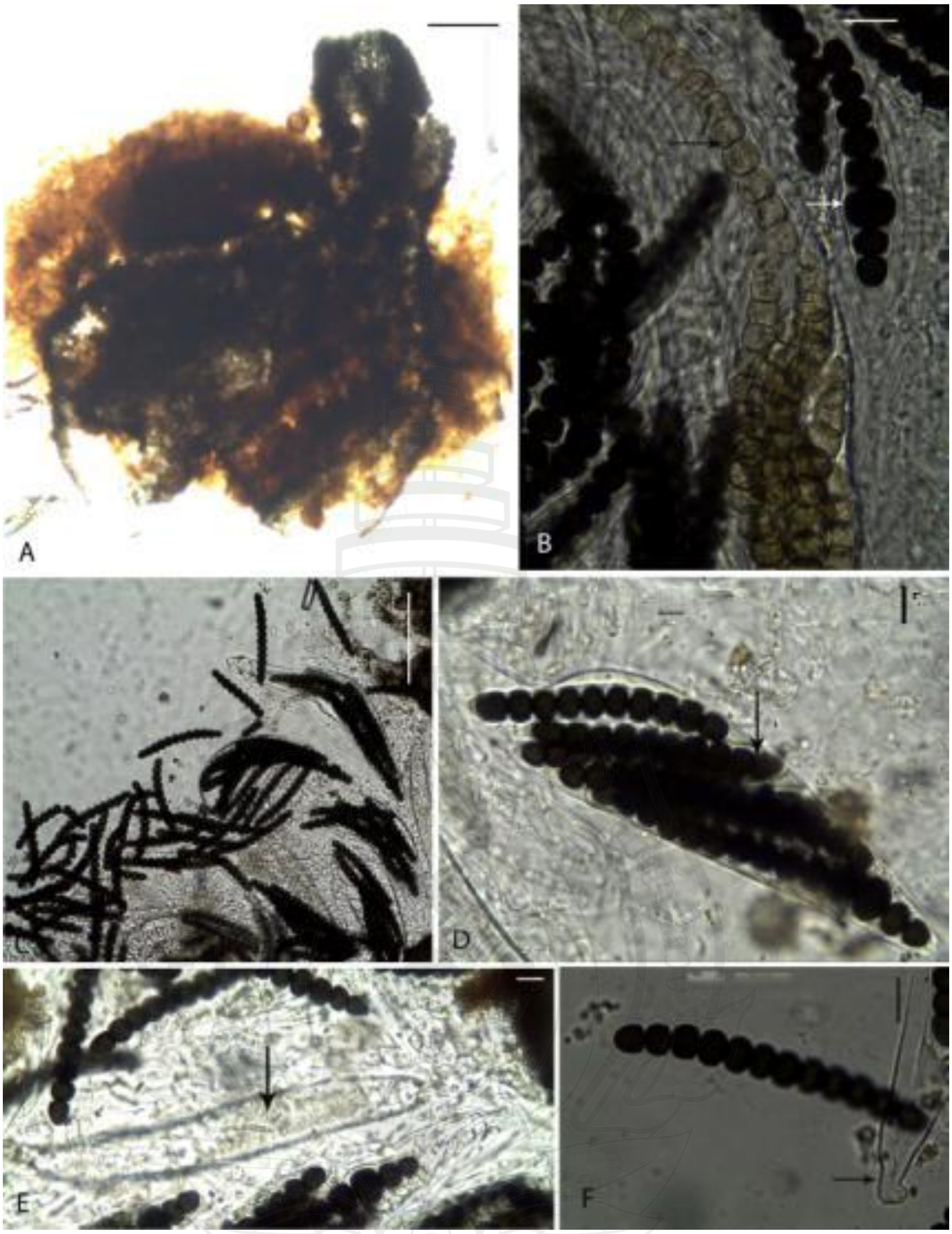
Since *Sporormiella* is composed of septate ascospores, the width of the ascospore was measured at the broadest and as near the middle section as possible and excluded the gelatinous envelope. To aid examination, some microstructures such as gelatinous sheath and spores were stained with lactophenol cotton blue and Congo red. Terminology used in this survey to describe germ slits and spore septation has been borrowed from Ahmed & Cain (1972) and Bell (2005).

8.3 Results and Discussion

The genus *Sporormiella* is characterized by immersed to semi-immersed scattered or gregarious ostiolate pseudothecia, with a membranaceous to coriaceous peridium dark brown to black, smooth in the upper part or covered with vestiture, with short papilliform or cylindrical neck. This genus shows a preference for growing on dung, has bitunicate/fissitunicate, cylindric-claviform 8-spored and stipitate asci. In addition, the ascospores are transversely to obliquely septate, at first hyaline finally becoming dark brown and surrounded by a common hyaline gelatinous sheath. The germ slit is usually elongated, extending the entire length of cell, parallel, diagonal or transverse to longitudinal axis (Cain, 1934; Ahmed & Cain, 1972; Bell, 1983, 2005; Doveri, 2004). The *Sporormiella* species examined are as described and illustrated in the following sections.

8.3.1 *Sporormiella herculea* (Ellis & Everh) Ahmed & Cain, Can J. Bot. 50: 442, 1972. (Figure 8.1 A-F)

Pseudothecia immersed, solitary, 890–950 µm high, 710–800 µm diam., glabrous, dark brown to almost black, subglobose. *Neck* relatively long, 250–400 µm, cylindrical, glabrous, black. *Peridium* layered to slightly coriaceous composed of *textura angularis* brown cells. *Pseudoparaphyses* filiform, abundant, septate, slightly longer than the asci and mixed with them, 4–7 µm broad. *Asci* 8-spored, 280–365 × 53–73.5 µm, clavate to slightly fusiform, broadly rounded above, slightly narrower near the upper end, broadest in the middle, contracting below into a persistent stalk 11.5–13 µm long. *Ascospores* obliquely bi- to tetra-seriate, 8–13-celled, 112–150 × 15–24 µm, cylindrical to slightly fusiform, hyaline when young, dark-brown at maturity; transversely septate, constrictions at septa broad and deep, segments not easily separating; third cell of each uppermost ascospore 16.5–19.5 × 22–25 µm, larger than the rest of the cells which are wider than they are long, end cells more rounded; *germ slit* transverse; *gelatinous envelope* narrow and hyaline.



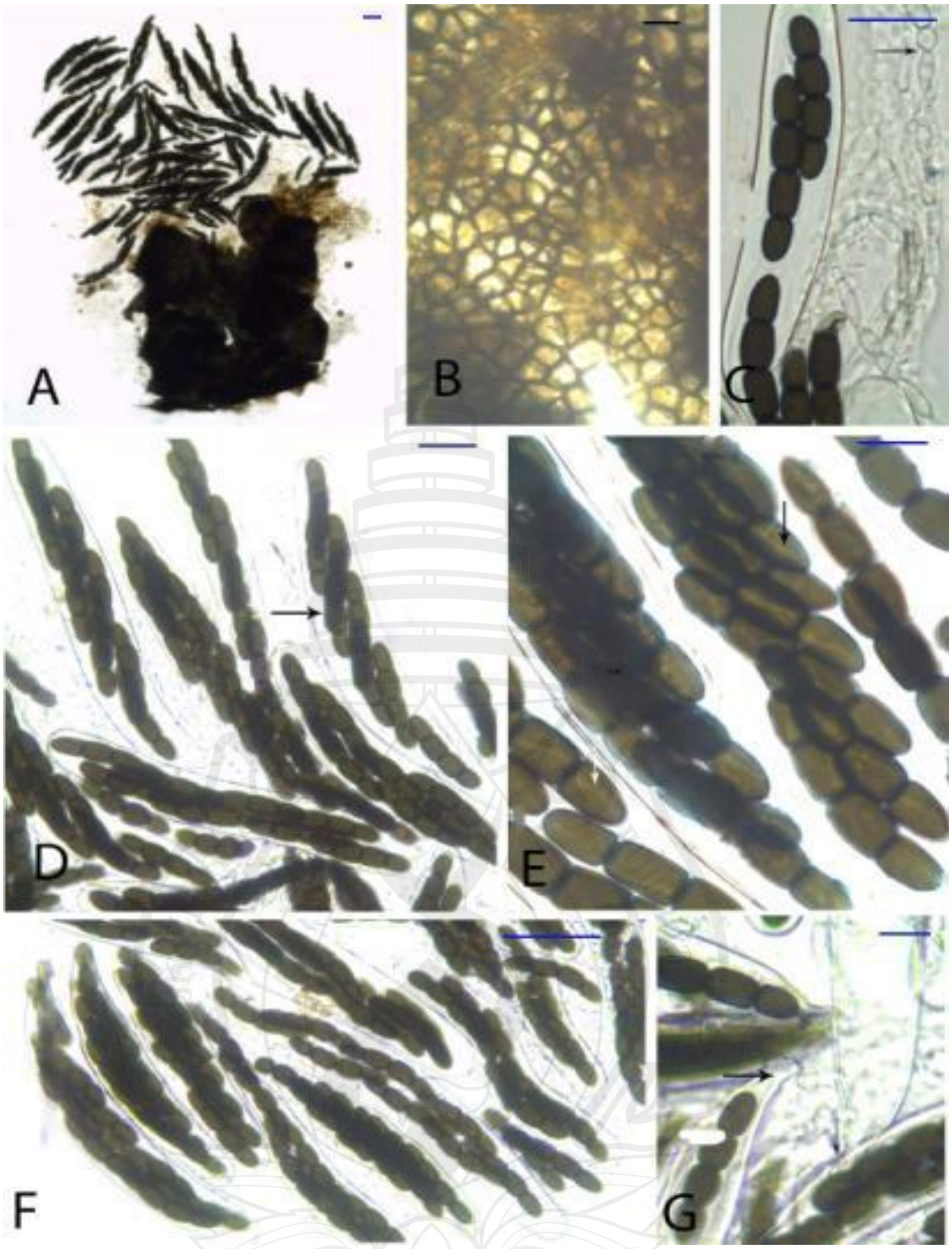
Note. (A) Squashed ascoma. (B) 8–13-celled hyaline ascospores (black arrow) among dark mature ascospores, note larger 3rd cell (white arrow) on an 8-celled uppermost ascospore. (C) Free mature dark celled ascospores. (D) Mature ascus with 10–12-celled ascospores amongst paraphyses. Note spore arrangement (arrow) and large 3rd cell on uppermost spore. (E) Immature ascus among free mature ascospores (arrow). (F) 12-celled ascospore and ascus stipe (arrow). **Scale bars**: (A, C) = 200 µm, (B, D-F) = 50 µm.

Figure 8.1 *Sporormiella herculea* (KWSNNP001-2009).

Sporormiella herculea is a highly variable species (Ahmed & Cain, 1972). This Kenyan specimen has 8–13-celled ascospores; the original diagnosis had 9–14-celled ascospores while another specimen from east Africa had 10–12-celled ascospores. The second to fifth cell of each uppermost ascospore is larger than the rest in all specimens (Cain, 1934; Ahmed & Cain, 1972; Doveri, 2004). A comparison of Canadian/USA specimens of *S. herculea* [spores (95–) 100–160 (–170) × 15–18 (–20) µm] with Brazil/Mexico [spores (87–) 90–125 (–140) × 15–18 µm] reveals some variability (Ahmed & Cain, 1972). Interestingly the Kenyan specimens put on the same scale [spores (100–) 126–154 × (12–) 15–17 µm (Ahmed & Cain, 1972) and 112–150 × 15–24 µm (personal observations in this study)] are closer in morphology to those from Canada and the USA implying that the variance is not necessarily based on a latitudinal gradient.

8.3.2 ***Sporormiella intermedia*** (Auersw.) S.I. Ahmed & Cain ex Kobayasi, in Kobayasi, Hiratsuka, Otani, Tubaki, Udagawa & Soneda, Bull. Natn. Sci. Mus., Tokyo 12:339, 1969. (Figure 8.2 A-F)

Pseudothecia immersed when young, superficial at maturity, scattered or in small groups, 200–370 µm high, 180–310 µm diam., smooth, dark brown to black, subglobose to pyriform; *neck* small, papillate, tips hyaline. *Peridium* membranaceous to semi-coriaceous, 3-layered; exostratum composed of thick-walled *textura-angularis* brown cells 5–9.5 × 6–8.5 µm; mesostratum composed of thick-walled elongated polygonal hyaline cells; endostratum composed of thin-walled elongated hyaline evanescent cells. *Pseudoparaphyses* filiform to moniliform, abundant, exceeding the asci and mixed with them, 5–6 µm diam., branched, septate, each segment more or less ovoid, constricted at the septa and broadest in the middle, containing numerous vacuoles, tips not swollen. *Asci* 8-spored, 140–190 × 22–31 µm, sub-cylindrical, broadly rounded above, broadest in the middle, abruptly contracting below into a stipe 15–20 µm long. *Ascospores* uniseriate in the upper and lower parts of ascus, usually obliquely biseriate in the middle, 4-celled, 44.5–56.5 × 8.5–10.5 µm, cylindrical, dark-brown and opaque at maturity, thick-walled, constrictions at septa broad and shallow, segments not easily separating, slightly curved; two middle cells almost equal in length and broader, terminal cells more tapered, slightly longer with more pointed apices; *germ slit* oblique and slightly sigmoid; *gelatinous sheath* broad, hyaline.



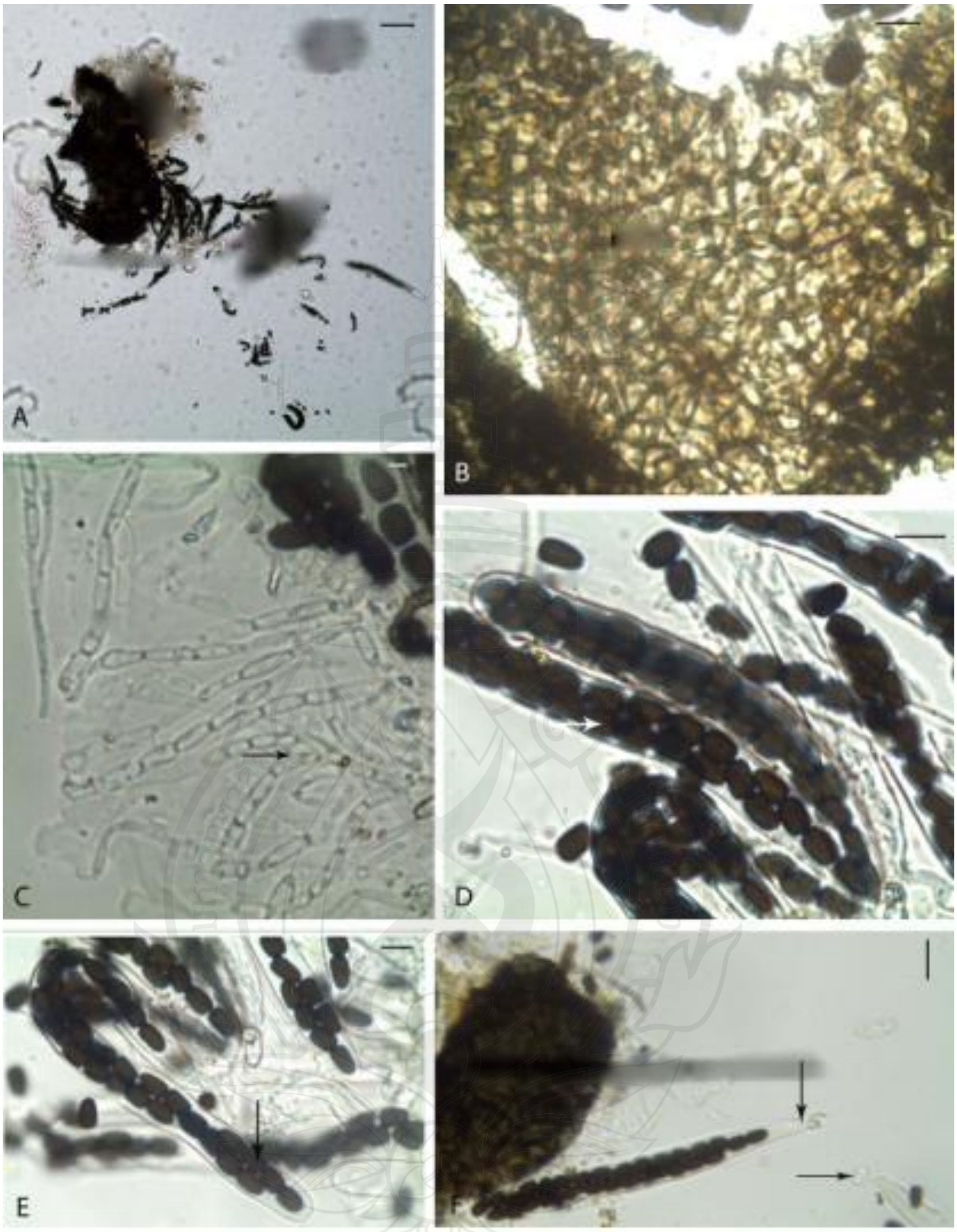
Note. (A) Squashed ascoma. (B) Details of exostratum. (C) Mature ascus with spores amongst moniliform paraphyses showing vacuoles (arrow). (D) Asci showing ascospore arrangement (arrow). (E) Ascospores showing germ slits (arrows). (F) Mature asci with ascospores (G) Asci tips showing stipes (arrow). Scale bars: (A) = 200 µm, (B-C, E) = 20 µm, (D, F-G) = 50 µm.

Figure 8.2 *Sporormiella intermedia* (KWSNNP008-2009).

Sporormiella intermedia is very closely related to *S. similis* R.S. Khan & Cain and *S. australis* (Speg.) Ahmed & Cain. This species has asci and spores intermediate in size between the two taxa. It has asci and spores that are smaller than those of *S. intermedia* but larger than *S. australis* (Doveri, 2004). According to Kruys & Wedin (2009) cylindrical asci with short stipes in combination with cylindrical spores circumscribe the *S. intermedia* clade composed of the closely related *S. australis*, *S. intermedia, S. lignicola* Phill. & Plowr, *S. minima* (Auesrw.) S.I. Ahmed & Cain, *S. similis, S. borealis* (I. Egel.) Krug, *S. bipartis* (Cain) Ahmed & Cain and *S. minipascua* Ahmed & Cain.

8.3.3 *Sporormiella leporina* (Niessl) S.I. Ahmed & Cain, Can. J. Bot. 50: 447, 1972. (Figure 8.3 A-F).

Pseudothecia immersed when young, semi-immersed at maturity, scattered or gregarious, 200–325 µm high, 165–250 µm diam., glabrous, brown to black, subglobose to pyriform; *neck* short, papilliform to cylindrical, rarely enlarged at the apex, straight or rarely with slight curvature, black. *Peridium* membranaceous to coriaceous, 2-layered; exostratum of thick-walled dark *textura angularis* cells, supporting septate brown hyphoid hairs; endostratum of small *textura angularis* cells. *Pseudoparaphyses* cylindric-filiform, abundant, septate, septa somewhat constricted, longer than asci and mixed with them, 2.5–3.5 µm broad, anastomosed, branched, segments cylindrical and nodulose, guttulate, tips not inflated. *Asci* 8-spored, 90–155 × 10.5–15.5 µm, cylindrical-clavate, tapering gradually downward from broadest part at apex into a fairly broad, lobate stipe 20–40 µm long. *Ascospores* obliquely biseriate above, uniseriate below, 4-celled, 32–36 × 5.5–6 µm, cylindrical, smooth, thick-walled, at first olivaceous brown, later dark-brownish, straight, rarely curved easily separating into single-celled units [Ahmed & Cain's (1972) material did not separate easily], septa constrictions broad and relatively shallow, upper terminal cell conical and notably narrowed to a tip (more pointed), middle cells oblong, lower terminal cell broadly rounded at the apex: germ slit oblique to nearly parallel; gelatinous sheath broad, hyaline.



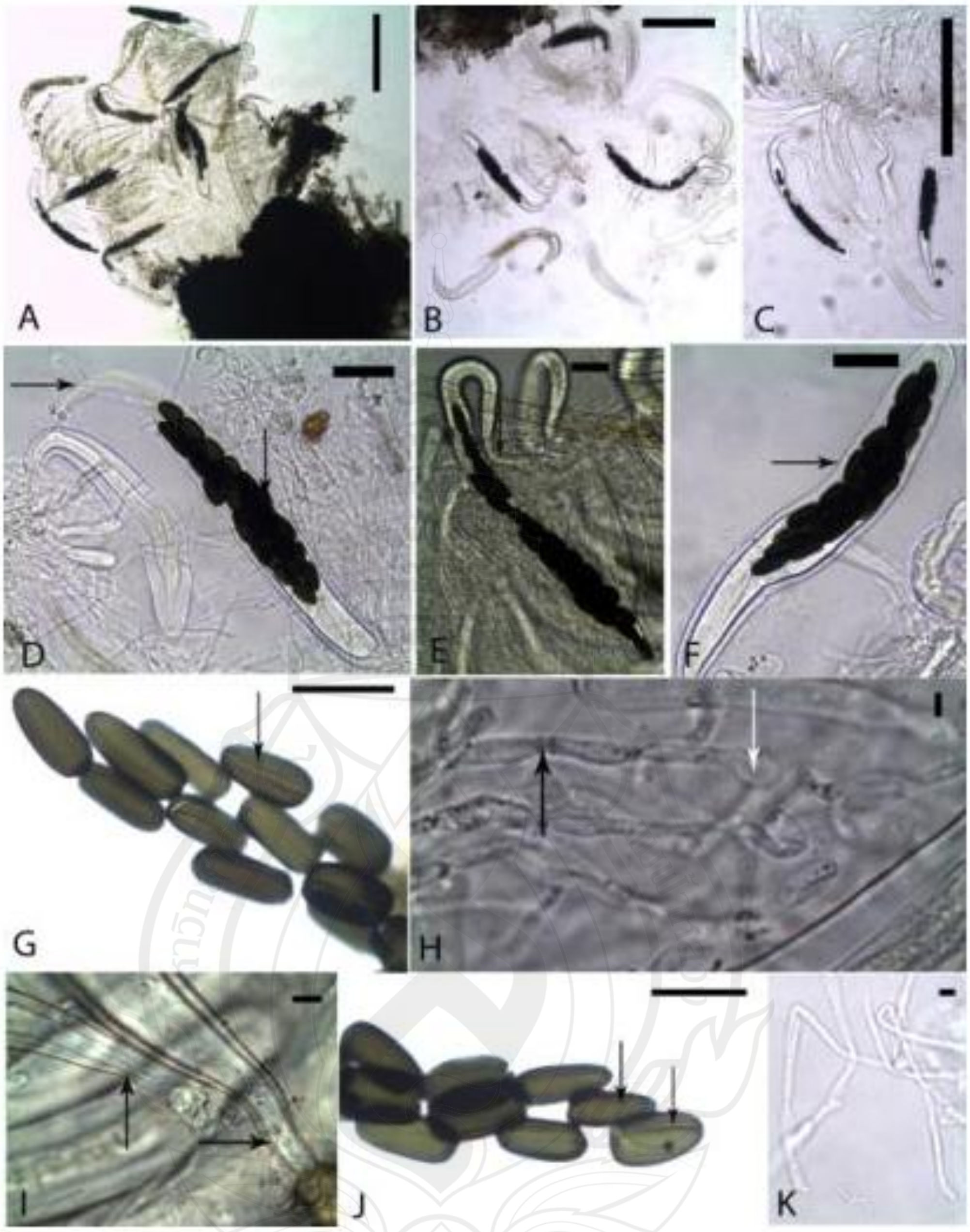
Note. (A) Squashed ascoma. (B) Details of exostratum. (C) Pseudoparaphyses. (D-E) Asci, note biseriate spore arrangement (arrow). (F) Asci stipes (arrows). Scale bars: (A) = 200 µm, (B-E) = 20 µm, (F) = 50 µm.

Figure 8.3 *Sporormiella leporina* (KWSSH001-2009).

Sporormiella leporina is very close to *S. isomera* Ahmed & Cain, however, it is differentiated by having a conical upper ascospore cell, second cell a little shorter, broader and rounder than the cylindrical third cell and an oblique germ slit (Ahmed & Cain, 1972; Doveri, 2004). The morphology of this specimen is close to *S. leporina* described by Ahmed & Cain (1972) and Doveri (2004). This Kenyan specimen has ascospores that separate easily when mounted in water slides. *Sporormiella leporina* is also related to *S. dakotensis* (Griffiths) Ahmed & Cain, *S. nigropurpurea* Ellis & Everh., and *S. subtilis* Ahmed & Cain belonging to a group with asci gently narrowing towards the bases and cylindric ascospores that are often less than 30 µm long (Doveri, 2004). *Sporormiella deserticola* (Faurel & Schoter) Doveri is differentiated from *S. leporina* by its cylindric asci, shorter stipe, larger ascospores (35–45 × 7–8 µm) and inconspicuous germ slits (Doveri, 2004). This is a new record for Kenya.

8.3.4 ***Sporormiella longisporopsis*** Ahmed & Cain, Can J. Bot., 50: 448–449, 1972. (Figure 8.4 A-K)

Pseudothecia immersed, scattered, 400–580 µm high, 300–500 µm diam., glabrous, dark brown to almost black, subglobose to pyriform; *neck* 100–200 µm long, cylindrical, bare, black. *Peridium* pseudoparenchymatous; exostratum of thin *textura angularis* brown polygonal cells. *Pseudoparaphyses* filiform, numerous, exceeding and mixed with the asci, 2–7 µm broad, sparingly branched, meshed, often anastomosed, containing hyaline vacuoles, tips sparingly inflated. *Asci* eight-spored, 280–380 µm × 35–43 µm, cylindrical-clavate, broadly rounded above, gradually contracting below into an elongated persistent stipe 16–29.5 µm long. *Ascospores* bi- or tri-seriate, 4-celled, 76–101 × 12–17 µm, cylindrical, initially olivaceous brown, later dark-brown and opaque, transversely septate; cells easily separable, single cells 23–32 × 12–14 µm; germ slit parallel; gelatinous sheath broad, hyaline.



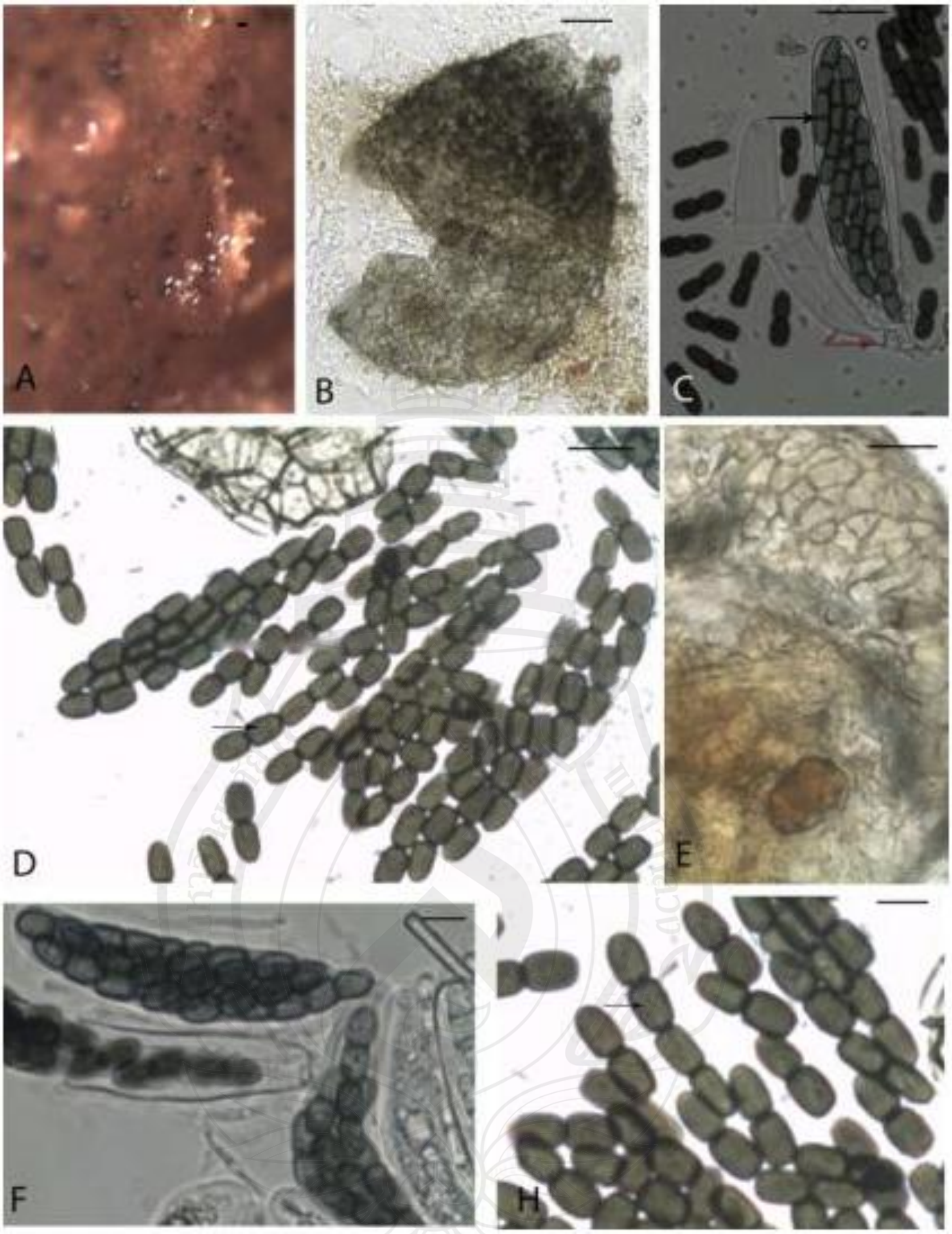
Note. (A) Squashed ascoma. (B-C) Mature and immature asci. (D) Mature and immature ascus, note spore arrangement and stipe (arrows). (E) Elongated asci stipe (arrow). (F) Ascus showing spore arrangement and tunica (arrow). (G, J) Mature ascospores showing germ slits (arrows). (H) Paraphyses showing septation (black arrow) and inflated tips (white arrow). (I) Asci stipes (arrows). (K) Paraphyses. **Scale bars:** (A, C) = 200 µm, (B, D, F) = 50 µm, (E, G-K) = 20 µm.

Figure 8.4 *Sporormiella longisporopsis* (KWSNNP017A-2010).

Sporormiella longisporopsis is hardly distinguishable from *S. longispora* (Ahmed & Cain, 1972; Doveri, 2004; Bell, 2005). Some important differences, however, exist; *S. longisporopsis* has oblique septa, parallel germ slit, more rounded spore cells, easy separability of spore segments, broad gelatinous sheath, wider spores and longer stipe, spores arranged obliquely with one at the highest level, then second, third, and fourth spore overlapping in succession inside the ascus. On the other hand, *S. longispora* has a shorter stout stipe, more pointed end cells, narrow gelatinous sheath and more or less rectangular ascospore cells with the uppermost 4 ascospores being almost at the same level inside the ascus (Ahmed & Cain, 1972; Doveri, 2004; Bell 2005). *Sporormiella longisporopsis* is a rare species having only being recorded previously from deer and hare dung in Japan (Furuya & Udagawa, 1972), giraffe dung in Kenya (Khan & Cain, 1979) and lagomorphs dung in Italy (Doveri, 2004).

8.3.5 *Sporormiella minima* (Auersw.) Ahmed & Cain, Can. J. Bot., 50: 419–447, 1972. (Figure 8.5 A-H)

Pseudothecia semi-immersed to nearly superficial at maturity, 95–160 µm high, 95–170 µm diam., scattered or loosely aggregated, dark brown to nearly black, smooth, subglobose to nearly pyriform. *Neck* 20–30.5 × 30.5–43 µm, papilliform to somewhat elongated, smooth, bare, dark-brown. *Peridium* thin pseudoparenchymatous. *Exostratum* thick-walled, composed of dark brown, *textura angularis* cells 8.5–13.5 × 7–12 µm. *Endostratum* consisting of pale thin-walled *textura angularis* cells. *Pseudoparaphyses* abundant, cylindric-filiform, 2–2.5 µm diam., septate, longer than the asci and mixed with them, simple, slightly swollen at the tips. *Asci* 8-spored, 73–148 × 13–20 µm, fissitunicate, broadly cylindrical and rounded above, widest in the middle, contracting abruptly into a short stipe 3.5–5 × 4.5–6.5 µm. *Ascospores* 4-celled, 27–33 × 4–7 µm, cylindrical, straight or curved, smooth, thick-walled, hyaline when young, becoming yellowish-brown, dark-brown when mature, gently and slightly tapering ends; uniseriate near the stipe, obliquely bi- or tri-seriate in the middle of ascus, transversely septate, septal constrictions broad, deep, cells more or less equal in size; terminal cells notably narrower towards their ends, cells readily separable at central septum, easily separable at other septa; germ slit almost parallel with a kink in the middle; gelatinous sheath hyaline, narrow.



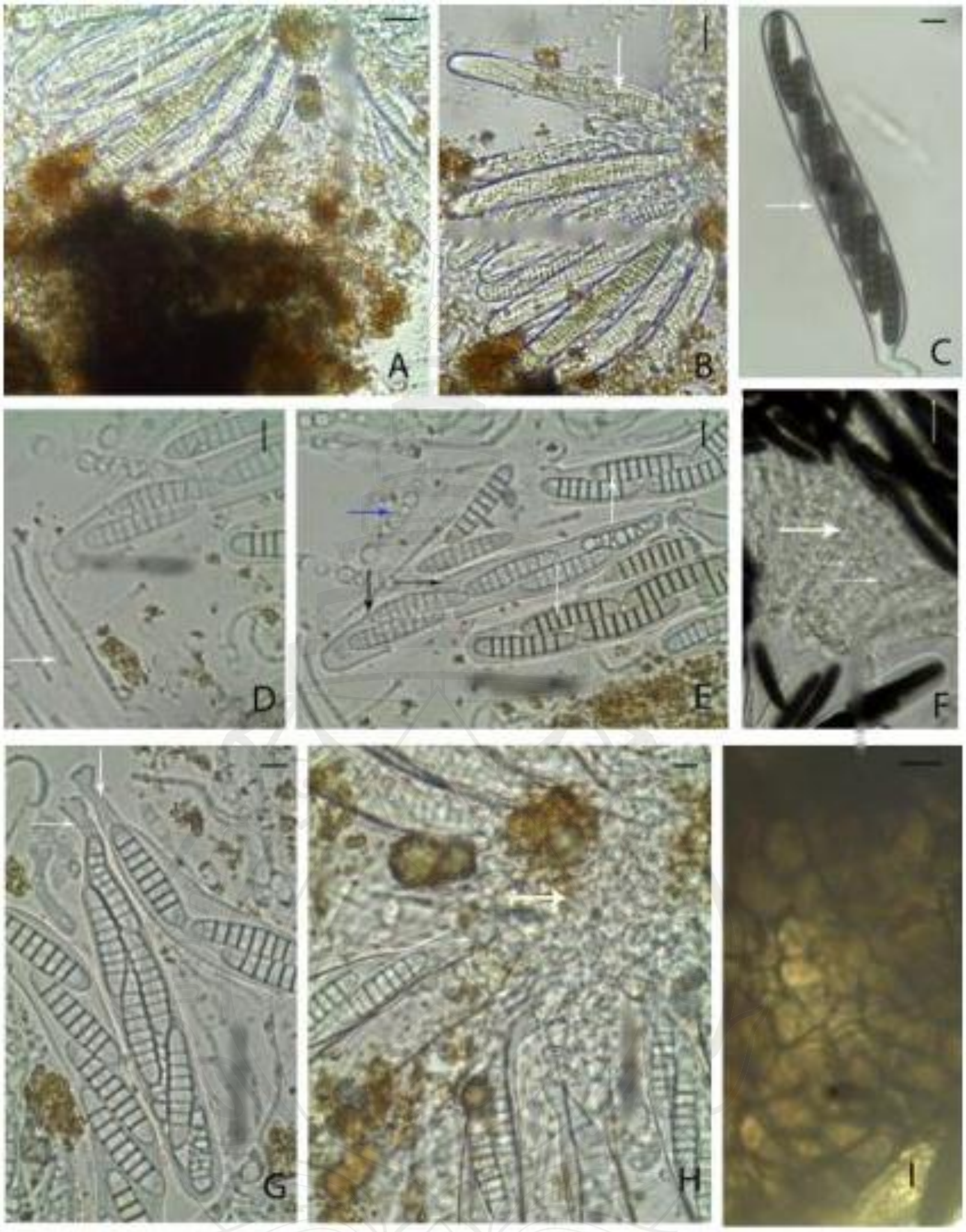
Note. (A) Ascoma on substrate. (B) Squashed ascoma. (C) Mature ascus, note spore arrangement (black arrow) and stipe (red arrow). (D, H) Mature ascospores, note germ slits (arrows). (E) Details of ascomatal wall. (F) Details of asci. Scale bars: (A) = 500 µm, (B) = 50 µm, (C-H) = 20 µm.

Figure 8.5 *Sporormiella minima* (KWSTE002A-2008).

Sporormiella minima is a very common species on dung (Furuya & Udagawa, 1972; Doveri, 2004).This taxon was observed many times across several dung types and was among the first to sporulate. The visible morphological characters fit those of *S. minima* from Australia and in the revised genera *Sporormia* and *Sporormiella* (Ahmed & Cain, 1972; Bell, 2005). *Sporormiella minima* is closely related to *S. minimoides* S.I. Ahmed & Cain (Doveri, 2004; Bell, 2005). However, *S. minimoides* has somewhat longer and broader spores with oblique or diagonal germ slits. It does not have a central kink and is not as commonly seen on dung as *S. minima* (Ahmed & Cain, 1972; Doveri, 2004). In addition, *S. minimoides* has a preference to grow on carnivore dung (Ahmed & Cain, 1972). This Kenyan collection has morphological features that agree with the descriptions of *S. minima* from Japan, Italy and Australia (Furuya & Udagawa, 1972; Doveri, 2004; Bell, 2005). This collection also has features that agree with the description of material examined by Ahmed & Cain (1972). This is a very common species on wildlife dung examined.

8.3.6 *Sporormiella* affinis *minipascua* S.I. Ahmed & Cain, Can. J. Bot., 50: 451, 1972. (Figure 8.6 A-I)

Pseudothecia immersed, scattered 200–250 µm diam., glabrous, dark brown to black, subglobose; *neck* short, cylindrical, glabrous, black. *Peridium* thin membranaceous to slightly coriaceous, composed of pale brown *textura angularis* cells, 4–12 µm broad, opaque. *Pseudoparaphyses* filiform, numerous, longer than and mixed with the asci, septate, fairly slender, with many prominent hyaline vacuoles, 3–6 µm diam., branched and anastomosed, tips not inflated. *Asci* 8-spored, 90–125 × 13.5–19 µm, cylindrical-clavate, broadest at the middle, rupturing in the middle section, contracting into a fairly long stipe. *Ascospores* obliquely bi- to tri-seriate above and in the middle, uni-seriate below, 8-celled, 27–33 × 5.5–7 µm, fusiform to cylindrical, brown at maturity, transversely septate, slightly curved, cells cylindrical, extreme end cells tapering, four middle cells similar in shape and size, cells not easily separable; germ slit oblique to almost diagonal; gelatinous sheath narrow, hyaline.



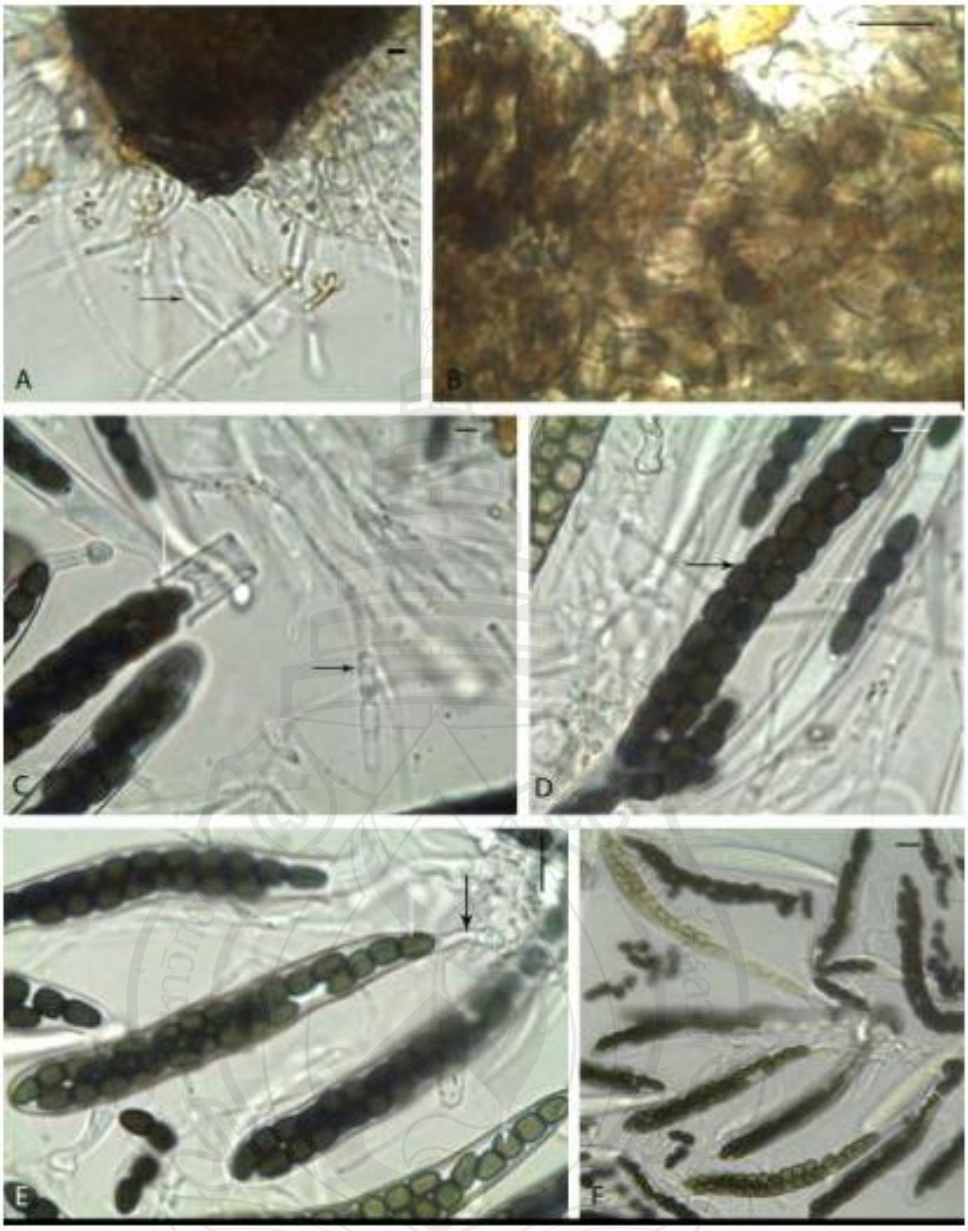
Note. (A-B) Part of a squashed ascoma, note 8-spored asci (white arrows). (C) Details of a mature ascus, note spore arrangement (arrow). (D-E) Free 8-celled ascospores (white arrows) among paraphyses, note the ruptured tunica (black arrows) and vacuoles (blue arrow). (F) Paraphyses showing septation (white arrows). (G) Asci showing stipes (arrows). (H) Centrum cells (arrow). (I) Details of exostratum. Scale bars: (A-B, F) = 50 µm, (C-E, G-I) = 20 µm.

Figure 8.6 *Sporormiella* affinis *minipascua* (KWSACC003-2009).

Sporormiella affinis *minipascua* is very close to *S. minipascua* and *S. pascua* (Niessl) Ahmed & Cain, however the former has a shorter stipe while the latter has larger ascospores (Ahmed & Cain, 1972). In addition, our Kenyan material has shorter and narrower spores than the original diagnosis of *S. minipascua*; 27–33 × 5.5–7 µm compared to 32–36 × 5.5–6.5 µm (Ahmed & Cain, 1972) and shorter but wider spores than the Japanese collection 27–35 × 5–6 µm (Furuya & Udagawa 1972). This specimen unlike *S. minipascua* has a long stipe and the fourth ascospore cell from the upper end does not seem to be the largest. This is probably a new species and record for Kenya.

8.3.7 *Sporormiella* affinis *muskokensis* (Cain) S.I. Ahmed & Cain, Can. J. Bot., 50: 451, 1972. (Figure 8.7 A-F).

Pseudothecia immersed, scattered, 300–500 µm high, 150–280 µm diam., membranaceous, smooth, slightly hairy, dark brown to blackish, subglobose; *neck* fairly long, cylindrical, bare, brown to black, 150–200 µm long, 50–100 µm diameter. *Peridial hairs* up to 90 µm × 1.5–3 µm. *Peridium* thin, membranaceous of *textura angularis* cells measuring 11.5–12 × 13–16 µm. *Paraphyses* filiform, numerous, branched, slightly longer than the asci and mixed with them, hyaline, septate, with numerous vacuoles, often nodulose. *Asci* 8-spored, 110–165 × 12.5–15.5 µm, cylindrical-clavate, broadly rounded above, tapering gradually from the broadest part near the apex into a relatively elongated, persistent stipe 10–15 µm long. *Ascospores* bi- to tri-seriate above, uniseriate below, 4-celled, 26–30.5 × 6–7 µm, cylindrical, straight to slightly curved, tapered, hyaline at first, then olivaceous to dark brown and opaque, easily breaking into 2-celled segments; septa transverse, broad and deep, 2 middle cells broader and almost equal in size, terminal cells markedly tapering and rounded at the ends with the upper one more pointed; germ slit parallel, occasionally diagonal and slightly sigmoid; gelatinous sheath narrow, hyaline.



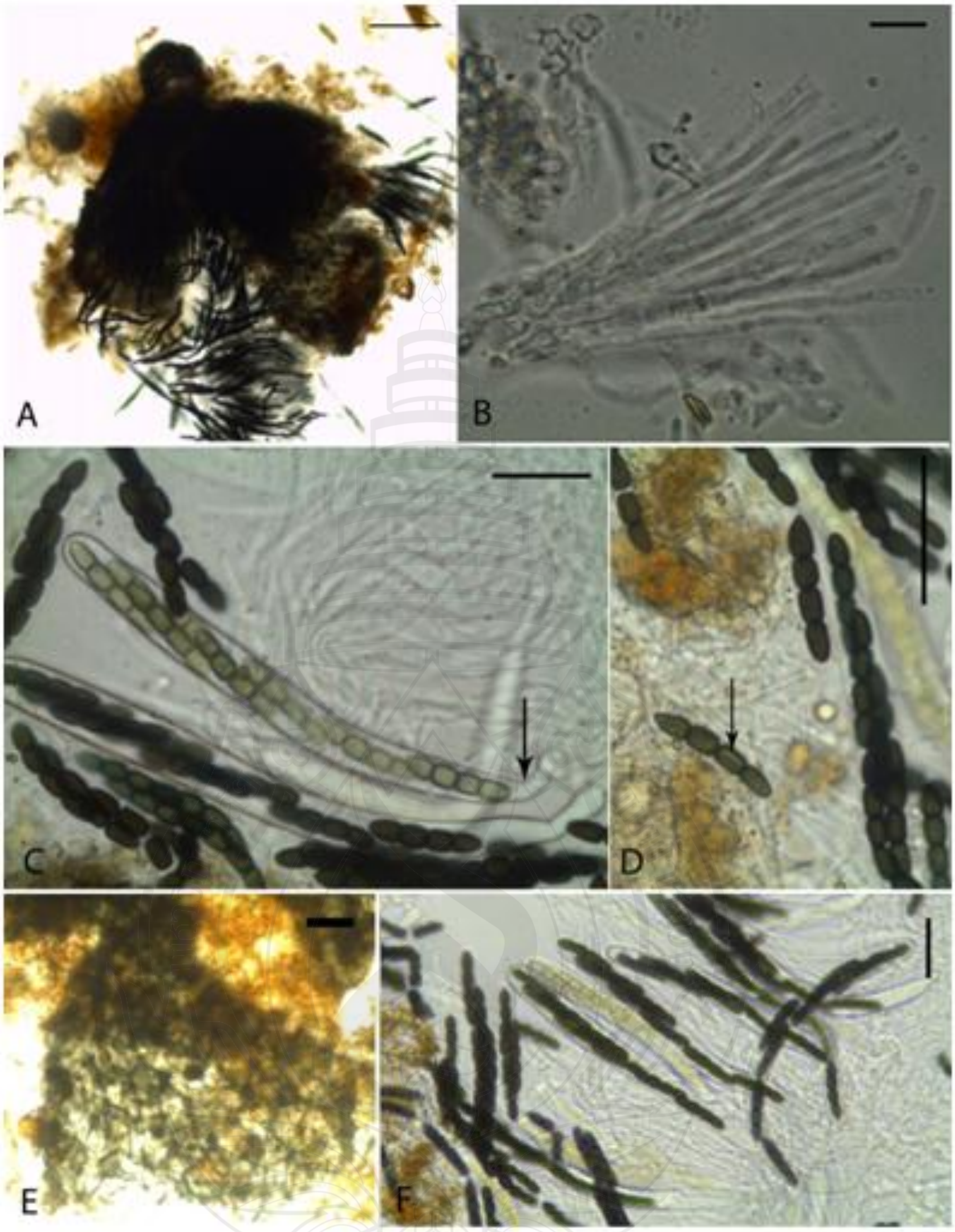
Note. A Peridial hairs (arrow). B Details of peridial wall. C Asci stipes, paraphyses (black arrow) and ruptured tunica (white arrow). D Mature 4-celled ascospores (arrows). E-F Mature ascus with ascospores, note long stipes (black arrow), spore arrangement and oblique germ slits (white arrows).Scale bars: A-E = 20 µm, F = 50 µm.

Figure 8.7 *Sporormiella* affinis *muskokensis* (KWSACC003-2009).

Sporormiella affinis *muskokensis* is close in many respects to *Sporormiella muskokensis*. The only slight difference is the transverse septa in the former. *Sporormiella* affinis *muskokensis* is close to *S. obliquisepta* Speg. [spores 37–42 × 7.5 – 8.0 µm, Cain (1934) and 37–42 × 7.5 – 8.5 µm, Ahmed & Cain (1972)] but the former is easily recognized by having narrower ascospores. It is also close to *S. obliqua* Khan & Cain from which it can be differentiated by the ascospore size (28–33 × 5.5–6.5 µm) and shape as well as type of germ slit (Cain, 1934; Ahmed & Cain, 1972; Khan & Cain, 1979; Doveri, 2004). *Sporormiella* affinis *muskokensis* is a new record for Kenya.

8.3.8 *Sporormiella* sp. Herbarium number KWSSH001-2009, giraffe dung. (Figure 8.8 A-G)

Pseudothecia semi-immersed to superficial, 350 µm high, 410 µm diam., scattered, dark brown to nearly black, glabrous, sub-spherical to pyriform. *Neck* short papilliform to cylindrical, 84 × 89.5 µm, [very long in the original diagnosis of the closely similar *S. dakotensis* (Ahmed & Cain, 1972)], larger on apical side, ascomal wall thick, membranaceous to coriaceous, consisting of dark brown, *textura angularis* of cells 7–10.5 × 3.5–6.5 µm. *Pseudoparaphyses* filiform, simple, septate, 70–90 µm long, 1.5–2 µm broad, anastomosed, tips not enlarged. *Asci* 115–159 × 8.5–11.5 µm, 8-spored, bitunicate, lacking an apical apparatus, cylindrical-clavate, broadest near the middle or below the apex, narrowly rounded in apical region, gradually contracted below into an elongated slender stipe 8.5–14 µm long [short in the original diagnosis of the closely similar *S. dakotensis* (Ahmed & Cain, 1972)]. *Ascospores* bi- or tri-seriate above and uniseriate below, 4-celled, 24.5–30 × 4–5.5 µm, notably tapered-cylindrical, olivaceous brown to dark brown, light green in reflected light when immature, transversely septate, deeply constricted and easily separable at septa into 2-celled units, cells oblong; terminal cells slightly narrowed and pointed at apices, upper ones more pointed, lower cell more pointed; germ slit parallel; gelatinous envelope hyaline, narrow.



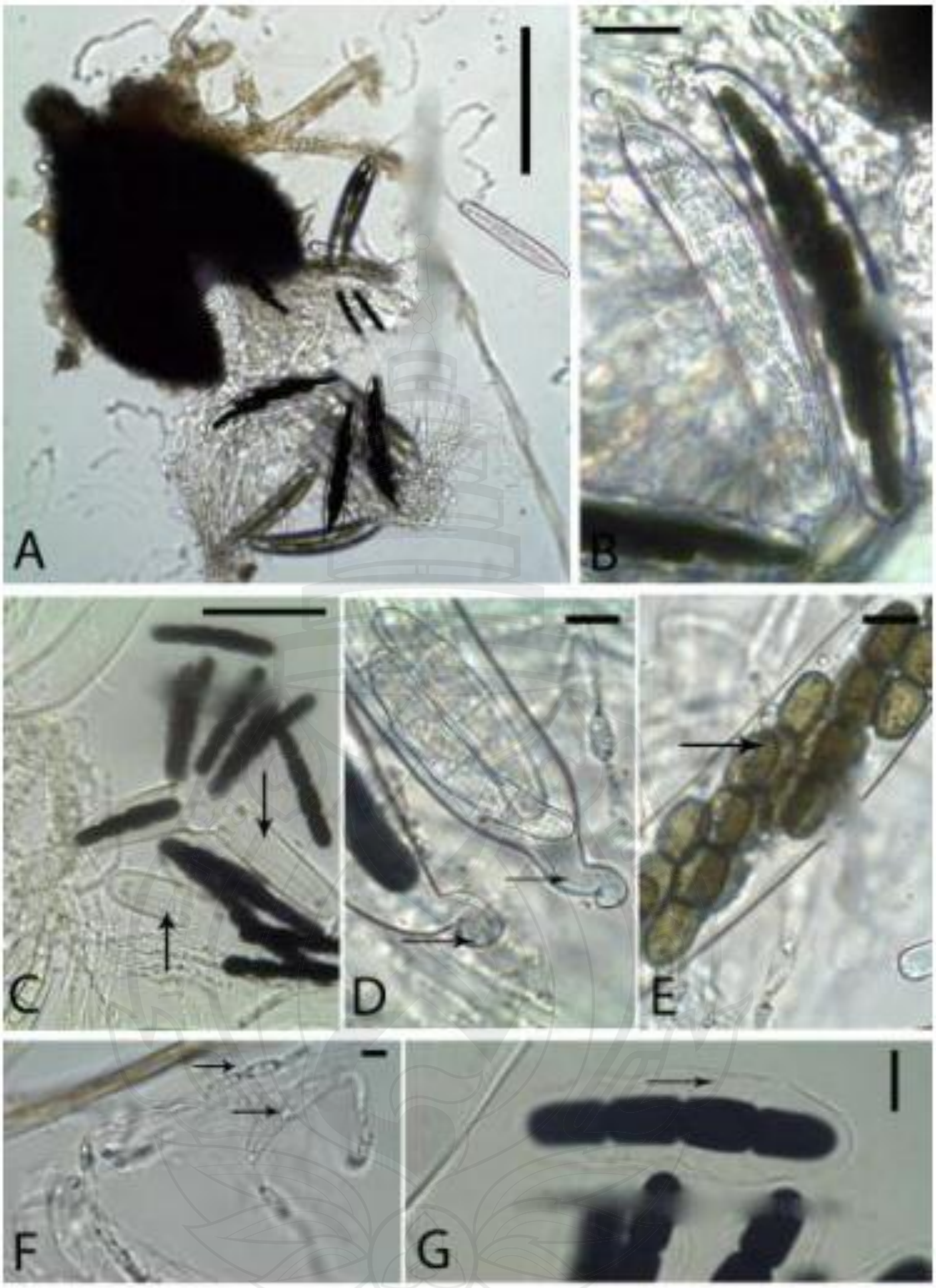
Note. (A) Squashed ascoma. (B) Pseudoparaphyses. (C) Asci showing stipe (arrowed) and spore arrangement. (D) Mature ascospores, note germ slit (arrowed). (E) Peridial wall. (F) Mature asci and spores. **Scale bars**: (A) = 200 µm, (B-C, E) = 20 µm, (D, F) = 50 µm.

Figure 8.8 *Sporormiella* sp. (KWSSH001-2009).

Sporormiella sp. compares closely with *S. dakotensis* described from Canada and Japan (Cain, 1934; Furuya & Udagawa, 1972). However, there are significant differences between the two species; Compared to *Sporormiella dakotensis*, this species has a shorter neck, longer and slender stipes, slightly longer asci and longer and broader obliquely uni- to tri-seriate ascospores (Cain, 1934; Ahmed & Cain, 1972; Doveri, 2004). *Sporomiella minutisperma*, a similar but independent species described by Doveri & Coué (2008b) has smaller ascospores. There is no species published after Ahmed & Cain's monograph that has features fully matching this specimen. This is probably a novel species and record for Kenya.

8.3.9 *Sporormiella* affinis *teretispora* S.I. Ahmed & Cain 1972 Can. J. Bot., 50: 460, 1972. (Figure 8.9 A-G)

Pseudothecia pyriform, superficial to semi-immersed, gregarious, 485–550 µm high, 340–400 µm diam., glabrous, almost smooth, pale brown; neck, 85 × 75 µm, cylindrical, smooth, hyaline. *Peridium* layered; endoperidium of pale, thin-walled polygonal cells; exoperidium of dark brown thick-walled polygonal cells. *Paraphyses* filiform, abundant, exceeding asci and mixed with them, 30–54 µm diameter, septate, containing many hyaline vacuoles. *Asci* 8-spored, 210–235 × 33–39 µm, cylindrical-claviform, roundish at apex, with a short stout slightly sigmoid stipe. *Ascospores* uniseriate in lower part of ascus, tri- to biseriate in the middle and upper part of ascus, 4-celled, 66–73.5 × 11–15.5 µm, cylindrical-fusiform, mostly straight to very slightly curved, not easily separating; middle cells almost rectangular, terminal cells more rounded; gelatinous sheath broad and serrated; germ slit diagonal, sigmoid to oblique.



Note. (A) Squashed ascoma. (B) Mature and immature asci. (C) Mature ascospores amongst dehisced ascus (arrows). (D) Asci showing stipes (arrows). (E) Immature ascus showing a triseriate spores arrangement (arrow). (F) Paraphyses showing septation (arrows). (G) Ascospore showing gelatinous sheath (arrow). Scale bars: (A) = 200 µm, (B-C) = 50 µm, (D-F) = 20 µm.

Figure 8.9 *Sporormiella* affinis *teretispora* (KWSACC002-2009).

Ascospores of *Sporormiella* affinis *teretispora* are 66–73.5 × 11–15.5 µm. *Sporormiella teretispora* has smaller cylindrical spores (60–) 63.5–68.5 (–69.5) × (10–) 11.5–12.5 (–13) µm (Ahmed & Cain, 1972) while *S. cylindrospora* has larger oblong-fusiform spores (67–) 70–80 (–90) × (13–) 15.5–16 (–17.5) µm (Cain, 1934; Ahmed & Cain, 1972; Doveri, 2004; Bell, 2005). *Sporormiella cylindrospora* and *S. teretispora* unlike this species have subglobose pseudothecia. Our Kenyan material has oblique triseriate spore arrangement and serrated gelatinous envelope different from *Sporormiella teretispora* and *S. cylindrospora*. *Sporormiella megalospora* has different spore shape with asci not abruptly contracted below. In view of this, the actual identity of this collection is not clear.

8.4 Conclusions

Sporormiella species obtained from dung of African elephant, Cape buffalo, dikdik, giraffe, impala and waterbuck incubated in a moist chamber were examined. Nine species namely, *Sporormiella herculea*, *S. intermedia*, *S. leporina*, *S. longisporopsis*, *S. minima*, *S. aff. minipascua*, *S. aff. muskokensis*, *Sporormiella sp*. and *S. aff. teretispora* were characterized and illustrated.

Sporormiella leporina, Sporormiella sp., *S.* aff. *minipascua, S. aff. muskokensis* and *S.* aff. *teretispora* are new records for Kenya. *Sporormiella minima* was the most common in the wildlife dung examined. *Sporormiella intermedia* and *S.* aff. *minipascua* were also fairly common on wildlife dung.

CHAPTER 9

COPROPHILOUS ASCOMYCETES: VII. *Chaetomium* Kunze

9.1 Introduction

Chaetomium belongs to the family Chaetomiaceae G. Winter (www.indefungorum.org/Names/Names.asp November, 2012). The family Chaetomiaceae was placed in the order Sordariales based on molecular studies (Lee & Hanlin, 1999). Species of this genus are very homogenous making intra-species differentiation very difficult. *Chaetomium* species grow on a variety of substrates such as plant remains, decomposing textiles, seeds, dried spices and sugar cane, with many species preferring materials with high cellulose content (von Arx et al., 1986; Doveri, 2004, 2008; Bell, 2005; Abdullah & Saleh, 2010). This genus *Chaetomium* was treated in detail by Dreyfuss (1976), von Arx et al. (1984), von Arx et al. (1986), Bell (2005) and Doveri (2008). Recent molecular phylogenetic studies (Lee & Hanlin, 1999; Greif et al., 2009) have helped to somewhat resolve the confusion in the systematics of Chaetomiaceae.

The genus *Chaetomium* is known to produce important secondary metabolites such as chaetomin, chaetoglobosins, cochliodinol sterigmatocystin, O-methylsterigmatocystin, chaetochromin, chaetocin and mollicellin G which have a wide range of bioactivity including antibacterial, mutagenesis, and toxicity and as natural dyes (Piecková, 2003).

Chaetomium has been recorded in many places around the world (von Arx et al., 1986; Bell, 1983, 2005; Doveri, 2004, 2008; Abdullah & Saleh, 2010). Records of *Chaetomium* in Kenya include *Chaetomium bostrychodes* Zopf, *C. murorum* Corda and *C. convolutum* Chivers, *C. apiculatum* Lodha, *C. aureum* Chivers, *C. caprinum*

Bainier, *C. coarctatum* Serg., *C. cochliodes* Palliser, *C. cuniculorum* Fuck., *C. dolichotrichum* Ames, *C. funicola* Cke, *C. globosum* Kunze, *C. indicum* corda, *C. multispirale* Carter, *C. quadrangulatum* Chivers, *C. retardatum* Carter & Khan, *C. subspirale* Chivers and *C. succineum* Ames, *C. crispatum* (Fuckel), *C. murorum*, *C. olivaceum* Cke & Ellis and *C. mareoticum* Besada & Yusef, *C. fusiforme* Chivers and *C. undulatum* Bainer (Minoura, 1969; Carter & Khan, 1982; Gatumbi & Kungu, 1994; Caretta et al., 1998). These studies depict a high diversity of *Chaetomium* species in Kenya.

The objectives of this survey were to examine and classify *Chaetomium* species from wild herbivore dung, document species diversity and distribution of *Chaetomium* species associated with dung from different wild herbivores in Kenyan National Parks and create awareness on role of fungi in conservation and management of biodiversity and the environment.

9.2 Materials and Methods

Seven dung samples collected from Kirk's dikdik, Jackson's hartebeest and impala, rarely studied substrates, were incubated and screened for *Chaetomium* as described in Section 2.2.

Morphological characters described in monographs and dichotomous keys on members of the genus *Chaetomium* were used for species circumscription (Dreyfuss, 1976; Bell, 1983, 2005; von Arx et al., 1984; von Arx et al., 1986; Richardson & Watling, 1997; Doveri, 2004, 2008). In *Chaetomium*, the shape and size of ascospores and terminal hairs were particularly important in highlighting differences amongst the species.

9.3 Results and Discussion

Chaetomium is characterized by superficial perithecioid, rarely cleistothecioid, ostiolate dark ascomata, usually adorning flexuous, spirally coiled or uncinate, rigid, straight or slightly curved setae and warted or verruculose, rarely smooth, wavy branched hairs over the entire ascoma and connected to the substrate by rhizoidal hyphae. Species of this genus have a relatively wide ostiolar pole whose apex is lined with periphyses and completely covered by terminal hairs making it almost obscure (von Arx et al., 1986; Doveri, 2004, 2008; Bell, 2005).

The relatively ephemeral asci develop in basal fascicles and are stalked, clavate, fusiform, obovate or narrow with a somewhat thin unitunicate evanescent wall. The ascospores are single-celled, pigmented, and pale when young, brown or grey olivaceous at maturity and have one or two distinct non-protuberant germ pores. Ascospores of most species are bilaterally flattened and are released from asci in a dark sticky cirrhus from whence they are dispersed in various ways including small animals and insects (von Arx et al., 1986; Bell, 2005; Doveri, 2008). Morphological characters of the terminal hairs, ascomatal wall, ascospore and cultural attributes are important diagnostic features for this genus.

Chaetomium is comprised of very similar species thereby making delimitation using morphological characters a very difficult task (von Arx et al., 1986; Bell, 2005; Doveri, 2008). Dreyfuss (1976) divided *Chaetomium* into ten basic groups but was only able to study four of these in detail, namely, the *C. murorum*, *C. globosum*, *C. bostrychodes* and *C. aureum* groups. These are based on the temperature and nutritional requirements, growth and fruiting body development rates, anamorph, compatibility, asci, ascospores shape and ontogeny. Ten isolates yielded the five taxa of *Chaetomium* described and illustrated in this chapter.

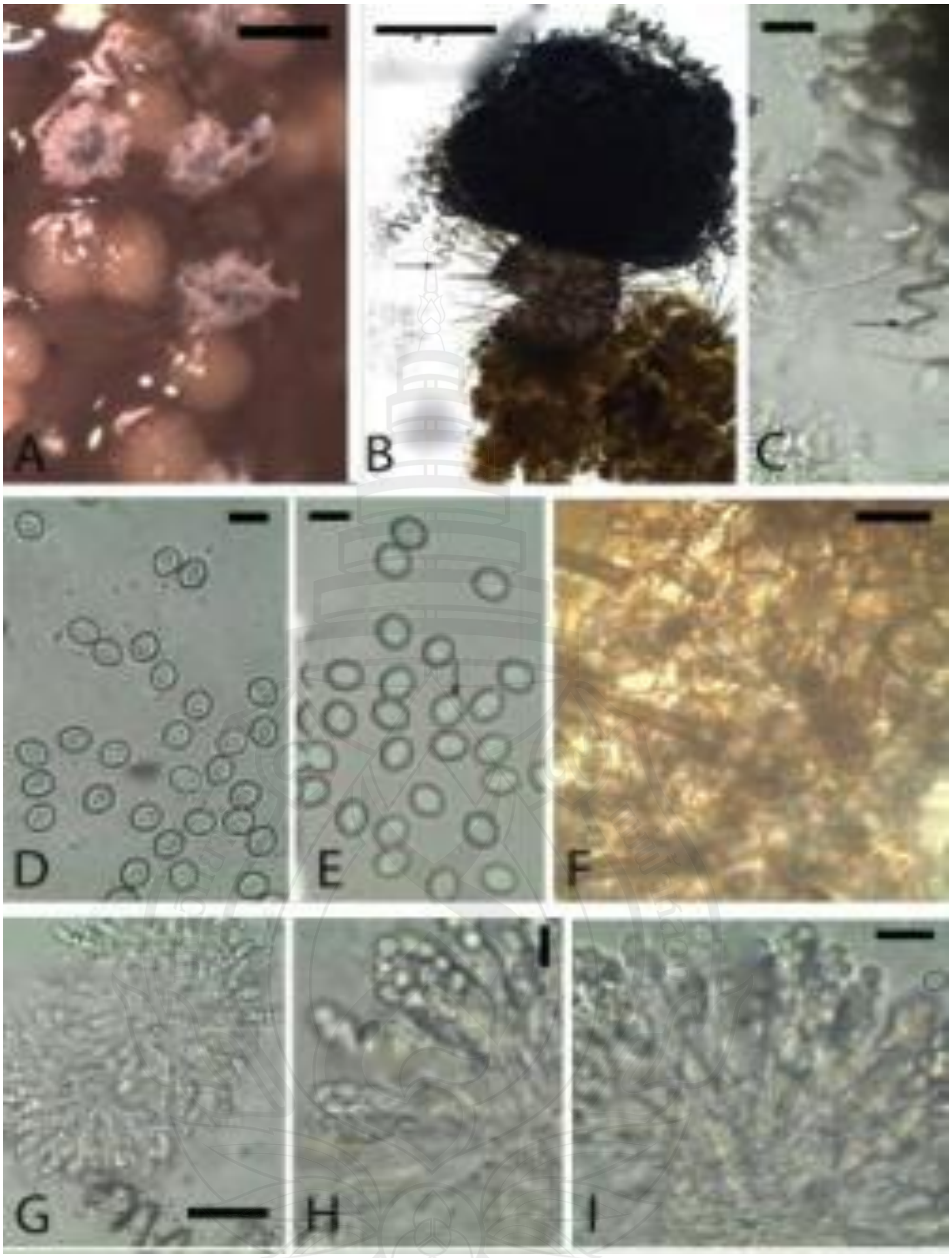
9.3.1 ***Chaetomium convolutum*** Chivers. Proc. Amer. Acad. 48: 85, 1912. (Figure 9.1 A-I)

Ascomata perithecioid, superficial, with well-developed dark brown rhizoids, 300–600 µm high, 100–225 µm diam., gregarious, pale to metallic or grey when young, obovate, ovoid, turbinate or ampulliform, ostiolate, ostiolar region wall often more darker. *Peridial wall* ochraceous or cinnamon-brown, elongate, *textura cephalothecoidea* thick-walled cells arranged in a petalloid structure around a hair bearing cell. *Terminal hairs* not branched, arising from the apical collar, erect, unevenly spiraled, thick walled, brown, septate, verrucose, the tips circinate; *lateral hairs* simple, up to 6 µm in diam., straight, tapering, bristle-like, septate, brown, warty. *Paraphyses* and interascal tissue not observed. *Asci* 8-spored, 34.5–54.5 × 9–12.5, unitunicate, thin walled, fasciculate, abundant, clavate, without an apical apparatus; stipe long, evanescent. *Ascospores* irregularly multi-seriate, 7–9.5 × 6.5–7.5 × 5–6.5 µm, limoniform to almost spherical, laterally flattened, single-celled, smooth, hyaline and dextrinoid when young, pale grey-bluish when mature, darker near the ends, without gelatinous equipment, bi-apiculate, germ pore apical.

Chaetomium convolutum belongs to the *C. bostrychodes* Zopf group. It closely resembles *Chaetomium bostrychodes* from which it is differentiated by the former's relatively larger ascospores with darkened ends and a darker *cephalothecoid* peridium (von Arx et al., 1986; Doveri, 2008). Intermediate species between *Chaetomium convolutum* and *C. bostrychodes* are frequently observed (von Arx et al., 1986).

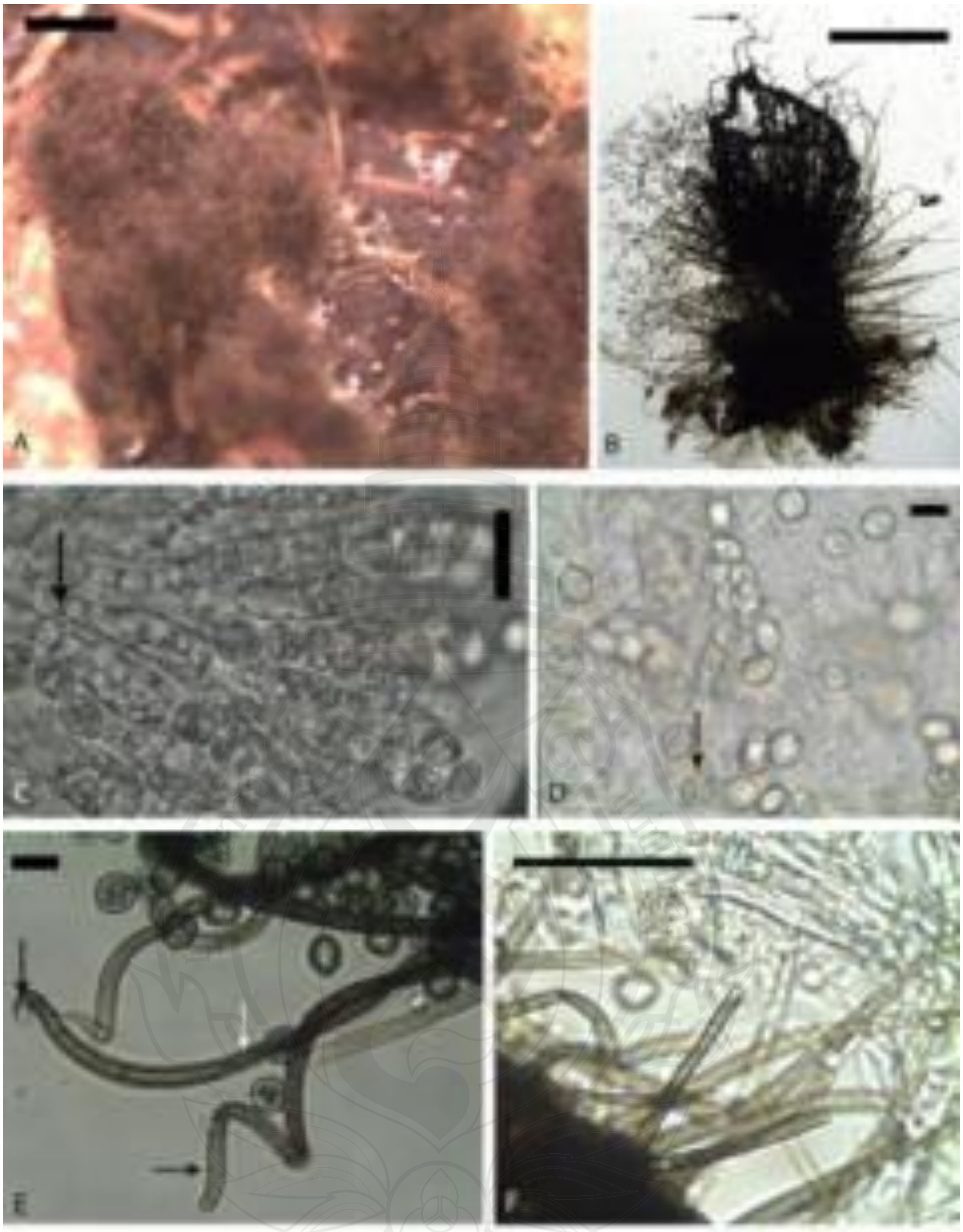
9.3.2 ***Chaetomium globosum*** Kunze:Fr. Syst. Mycol. Soc. 44:46, 1961. (Figure 9.2 A-F)

Ascomata perithecioid, superficial, with brown rhizoids, 150–250 µm diam., 400–700 µm high (including terminal hairs), olivaceous to grayish-green, fully covered by vestiture, with yellowish exudates, gregarious, spherical to ovoid or obovate, ostiolate. *Peridial wall* brown of *textura intricata* of cells 2–3.5 µm broad. *Terminal hairs* numerous, simple, stiff lower parts, flexuous, undulate or irregularly coiled, tapering, septate, greenish-brown, strongly verrucose, those covering upper part of ascoma 4.5–6.5 µm in diam. at the base. *Lateral hairs* septate, tapering, warty, 3.5 × 84 µm in diam., hardly differentiated from the apical hairs. *Paraphyses* highly evanescent at an early stage, exceeding the asci and mixed with them, filiform, septate. *Asci* ephemeral, 8-spored, 51–77 × 11.5–15.5 µm, clavate or slightly fusiform, evanescent at an early stage, thin unitunicate wall, lacking an apical apparatus; stipe up to 28 µm long. *Ascospores* 9.5–11 × 7 – 9.5 × 6 – 8 µm, irregularly biseriate, limoniform, usually weakly bi-apiculate, bilaterally flattened, fairly thick walled, smooth, brownish when mature, spotting numerous guttules, lacking a gelatinous sheath; single small apical germ pore.



Note. (A) Ascomata on dung. (B) Squashed ascoma, note uneven spiraling (arrow). (C) Terminal hairs, note uneven spiraling (arrow). (D-E) Mature ascospores, note apical germ pore (arrow). F Ascomatal wall, note the petalloid structure (arrow). (G-I) Asci at various stages of development. Scale bars: (A) = 500 µm, (B) = 200 µm, (C, G) = 50 µm, (D-F, H-I) = 20 µm.

Figure 9.1 *Chaetomium convolutum* (KWSTE005B-2008).



Note. (A) Ascomata on dung. (B) Squashed ascoma, note coiling of terminal hairs. (C) Mature asci, note stipe. (D) Free mature bi-apiculate ascospores, note germ pore (arrow). (E) Terminal hairs showing coiled tips and warty surfaces (arrows). (F) Lateral hairs. Scale bars: (A) = 500 µm, (B) = 200 µm, (C-F) = 20 µm.

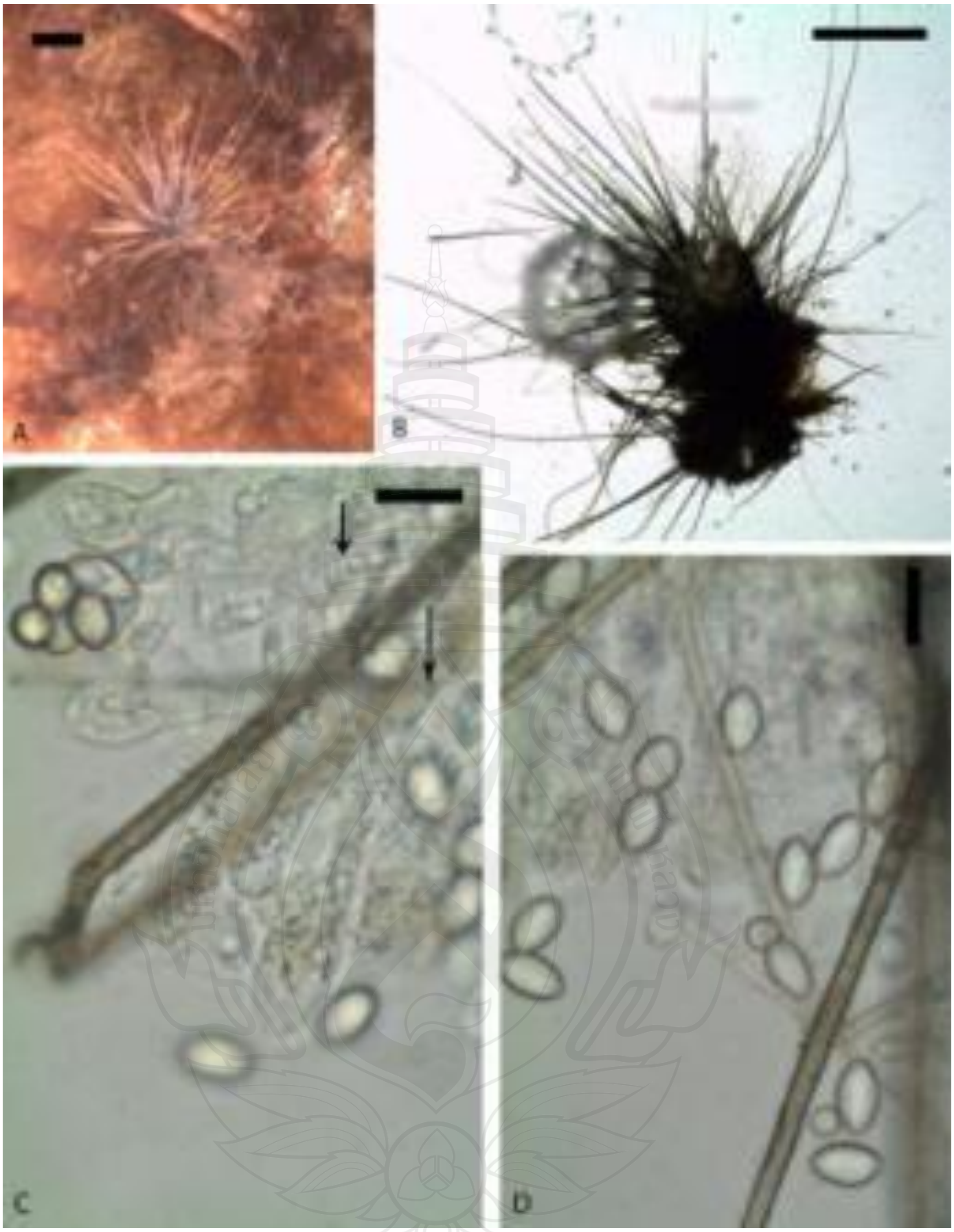
Figure 9.2 *Chaetomium globosum* (KWSNNP013-2009).

Chaetomium globosum is a very common and variable species. Strains of *Chaetomium globosum* possessing spiraled terminal hairs are historically identified as *C. cochliodes* and large-spored variants treated as *C. olivaceum*. Strains sometimes considered as close relatives are heterothallic, often expressing in culture as immature ascomata with retarded growth and development (Dreyfuss, 1976; von Arx et al., 1986). *Chaetomium globosum* is usually isolated from soil, rotting vegetation, cellulosic products including paper and is also commonly encountered in indoor environment fungi surveys (Dreyfuss, 1976). *Chaetomium globosum* strains are diagnosed through the variability in colony pigmentation and colour of ascomatal hair in reflected light (von Arx et al., 1986; Doveri, 2008). The differences in pigment and colour which ranges between pale green to ochraceous with some even being dark olivaceous are used to circumscribe the strains (von Arx et al., 1986; Doveri, 2004, 2008). *Chaetomium globosum* is similar to: *C. elatum* Kunze but can be differentiated by an ascomata that develops slowly plus dichotomously (regularly) branched terminal hairs; *C. subaffine* Serg. differentiated by its larger ascospores and flexuous, straight and longer ascomatal hairs; *C. coarctatum* differentiated by its slower ascomata development and *C. spirochaete* which has dark regularly coiled terminal hairs (von Arx et al., 1986; Doveri, 2004, 2008).

9.3.3 *Chaetomium muelleri* Arx, in von Arx, Guarro & Figueras, Beih. Nova Hedwigia 84: 6, 1986. (Figure 9.3 A-D)

Ascomata perithecioid, superficial, 100–320µm high, 80–185 µm diam., scattered, olivaceous, ovate, ostiolate, exudate not prominent, with white or grayish to pale aerial mycelium, hairs not spirally coiled. *Ascomatal wall* composed of *textura angularis/petalloid* brown or pale cells. *Terminal hairs*, actuate or flexuous, dark brown, verrucose, over 900 µm long, 3–7 µm broad, sparingly septate: tips pale or hyaline, straight. *Lateral hairs* seta-like up to 13 µm diam. at base, verrucose, septate. *Paraphyses* not observed. *Asci* 8-spored, 38.5–65.5 × 14.5–16.5 µm, clavate, unitunicate, thin non-persistent wall, long stipitate. *Ascospores* 10.5–13.5 × 7–8 µm, irregularly multiseriate, ellipsoid-fusiform, with rounded ends, smooth, grayish-brown at maturity, lacking gelatinous appendages or sheath; germ pore protuberant, slightly sub-apical.

Chaetomium muelleri is close to *C. carinthiacum* Sorgel, however the latter has smaller ascospores and paler thinner ascomatal hairs (von Arx et al., 1986). The ascospores of *C. muelleri* are similar in size to those of *C. raii* Malhotra & Mukerji. However, the latter is differentiated by the ascomatal shape and flexuous spirally coiled hairs (Doveri, 2008). *Chaetomium muelleri* is a new record for Kenya.

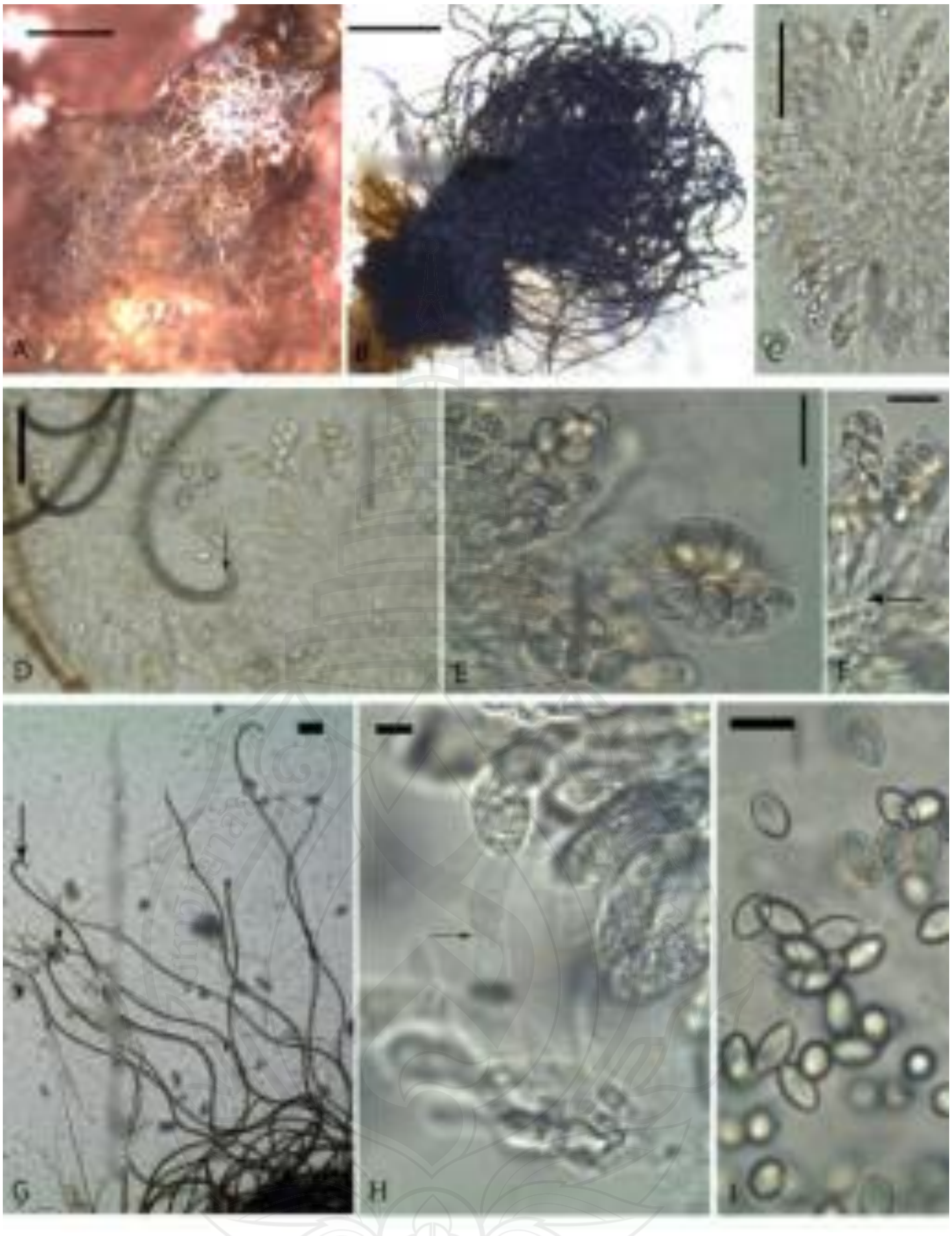


Note. (A) Ascomata on dung. (B) Squashed ascoma, showing long straight terminal hairs. (C) Asci showing long stipes (arrows). (D) Free mature ascospores. Scale bars: (A) = 500 µm, (B) = 200 µm, (C-D) = 20 µm.

Figure 9.3 *Chaetomium muelleri* (KWSACC004-2009).

9.3.4 *Chaetomium seminis-citrulli* Sergejeva, Not. Syst. Sect. Crypt. Inst. Bot. Acad. Sci. U.R.S.S. 11: 113, 1956. (Figure 9.4 A-I)

Ascomata perithecioid, superficial, 800–1000 µm high (including hairs),150–290 µm diam., gregarious, dark-gray in reflected light, ostiolate, globose, fully covered by hairs, with white aerial mycelium. *Peridial wall* very thin, dark brownish. *Ascomatal hairs* numerous, wavy, thick-walled, over 900 µm long, unbranched, straight and bulbous at the base, apically circinate, indistinctly septate, grey, dark brown, almost smooth to verruculose, 4–8 µm broad in middle parts. *Paraphyses* moniliform, hyaline, few, 6–8 µm broad, very evanescent, septate. *Asci* 8-spored, 63.5–84.5 × 16–21 µm, unitunicate, thin walled, evanescent, claviform, long stipitate. *Ascospores* 12.5–14 × 7.5–8.5 µm, irregularly biseriate, bilaterally flattened, broadly ellipsoidal, somewhat thick-walled, with rounded ends, rather symmetrical, smooth, olivaceous or pale grayish at maturity, with an indistinct apiculus on one end and a distinct germ pore at the attenuated end.



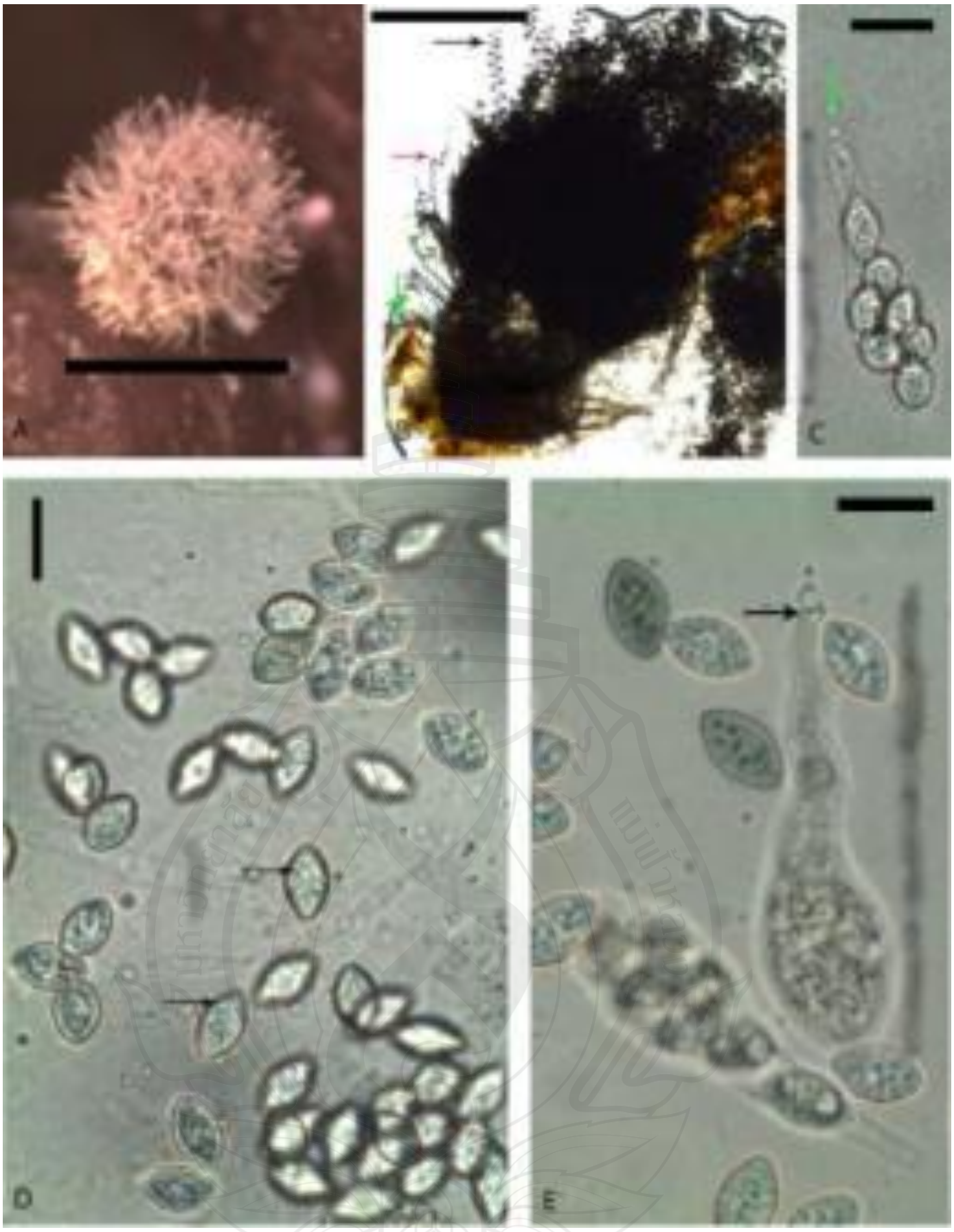
Note. (A) Ascomata on dung. (B) Squashed ascoma. (C) Asci. (D) Terminal hairs and asci, note curved apices (arrow). (E- F) Mature asci and ascospores, note stipe (arrow). (G) Terminal hairs showing curved apices (arrows). (H). Paraphyses, note septation (arrow). (I) Free mature ascospores. Scale bars: (A) = 1000 µm, (B) = 200 µm, (C-E) = 50 µm, (F-J)= 20 µm.

Figure 9.4 *Chaetomium seminis-citrulli* (KWSACC004-2009).

Chaetomium seminis-citrulli ascospore size and shape resemble those of *C. elatum* and *C. gangligerum* Ames but the size of asci is notably different (von Arx et al., 1986). It is related to *Chaetomium crispatum* which has smaller asci and ascospores (von Arx et al., 1986). *Chaetomium seminis-citrulli* is also close to *C. vitellinum* Carter from which it is differentiated by having smaller bilaterally flattened ascospores (Doveri, 2004). The spore size range and asci width of *Chaetomium seminis-citrulli* falls within the limits of *C. succineum*, the only difference being the asci length (von Arx et al., 1986). According to Doveri (2004, 2008) *Chaetomium seminis-citrulli* is rare and has only been observed in USSR, Israel and Italy.

9.3.5 *Chaetomium* sp. from dikdik dung, herbarium number KWSACC004-2009 (Figure 9.5 A-E)

Ascomata perithecioid, superficial, 400–620 µm high, 100–270 µm diam., solitary or gregarious, spherical or ovate, with light greenish exudates, with white aerial mycelium. *Peridial wall* pale brown, composed of *textura angularis* thick-walled cells. *Terminal hairs* numerous, 3–5.5 µm broad, flexuous, with even spirals, variable length, septate, verrucose, white to pale green. *Lateral hairs* fewer, 5–6 µm broad at base, up to 90 µm long, brown, septate, verrucose; tips coiled or straight. *Paraphyses* scanty, moniliform, 3.5–7.5 µm broad, septate hyaline, with numerous vacuoles. *Asci* 8-spored, 58–61.5 × 15.5–18 µm, broadly clavate to obovate, evanescent, with slender stipes 10–17 µm long. *Ascospores* 10.5–14 × 6.5–8 µm, irregularly biseriate, ellipsoidal-fusiform, symmetrical, attenuated and umbonate at both ends, brown when mature, with many oil droplets, bi-apiculate.



Note. (A) Ascoma on dung. (B) Squashed ascoma, note even spiraled terminal hairs (black arrow), lateral hairs tips (red and green arrow). (C) Ascus, note ascospore arrangement and stipe (arrow). (D) Free mature ascospores amongst paraphyses. (E) Immature asci amongst free ascospores, note long stipe (arrow). Scale bars: (A) = 500 µm, (B) = 200 µm, (C-D, E) = 20 µm.

Figure 9.5 *Chaetomium* sp. (KWSACC004-2009).

Chaetomium sp. appears to be close to *C. gracile* and *C. gangligerum*; the ascospore size is within the range for *C. gracile* and *C. gangligerum*; the width of the asci of our collection is also similar to that of *C. gracile* and *C. gangligerum*; however, the length is markedly different (von Arx et al., 1986; Doveri, 2008). Another notable difference is the evenly spiraled terminal hairs in our specimen. This collection is also similar to *Chaetomium raii* (von Arx et al., 1986; Doveri, 2008) but has slightly smaller ascospores and wavy terminal hairs (von Arx et al., 1986).

9.4 Conclusions

Five species, *Chaetomium convolutum*, *C. globosum*, *C. muelleri*, *C. seminis-citrulli* and *Chaetomium* sp. were examined, described and illustrated. *Chaetomium seminis-citrulli* was a new records. The most common species were *Chaetomium convolutum* and *C. globosum.*

Most of the *Chaetomium* species observed in this study show a preference to grow on dung of browsing wild herbivores.

CHAPTER 10

OVERALL CONCLUSIONS

Kenya is home to numerous species of wild animals that comprise diverse species of arthropods, reptiles, birds and mammals. Animal dung is a nutrient-rich substrate for saprobic fungi. The ecology and taxonomy of coprophilous ascomycetes from most livestock have been undertaken in Europe and North America. Ascomycetes from dung of the vast range of African wildlife has not been examined, with only a handful of wild animals being sampled for study. This survey was done with an objective of assessing the biodiversity and taxonomy of coprophilous ascomycetes occurring in wildlife dung in Kenya.

10.1 Diversity of Coprophilous Ascomycetes

Moist chamber cultures of Kenyan wildlife dung yielded ten genera represented by 42 species of coprophilous ascomycetes as follows: six species of *Ascobolus* seven of *Saccobolus*, five of *Schizothecium*, five of *Podospora*, eight of *Sporormiella*, five of *Chaetomium*, one of *Arnium*, two of *Zopfiella*, one of *Zygopleurage* and one of *Sordaria*.

We sampled 13 wild animal species from Aberdare Country Club Game Sanctuary and Aberdare National Park, Kinondo Forest Reserve, Nairobi National Park, Shimba Hills National Reserve and Tsavo East National Park.

Type, structure, texture, moisture content and age of dung collected are important variables for the occurrence of coprophilous *ascomycetes*. These variables are highly dependent on animal species voiding it. The age of dung at sampling and the time taken from sampling to incubation had a notable influence on the composition of ascomycetes sporulating with most of the early sporulating species being less common on old or preserved dung. Impala, giraffe, dikdik, waterbuck and elephant dung from animal species exhibiting different feeding habits, had the highest number of specimens and species of ascomycetes.

Species of the genus *Ascobolus* were noted to sporulate only on dung that was cultured immediately after sampling. Probably preservation has an influence on ascospore viability for this genus.

10.2 New Species and new Records of Coprophilous Ascomycetes

We used morphological characterization to classify coprophilous ascomycetes in this survey. We were able to describe two new species, 16 new records and six undetermined species of coprophilous ascomycetes from the dung samples screened.

The new species described include *Ascobolus nairobiensis* sp. nov. and *Ascobolus tsavoensis* sp. nov. New records were *Schizothecium conicum, S. dubium, S. glutinans*, *Chaetomium seminis-citrulli, Sporormiella leporina, Podospora minor, Ascobolus bistisii, Ascobolus calesco, Saccobolus citrinus, S. diffusus, S. infestans, S. platensis, S. truncatus, Arnium arizonense, Zopfiella longicaudata* and *Sordaria fimicola.*

Based on the existing dichotomous keys, some taxa could not be placed and were treated as undetermined species. These were *Sporormiella* aff. *minipascua, S.* aff. *muskokensis*, *Sporormiella* sp. and *Sporormiella* aff. *teretispora, Zopfiella* aff. *erostrata* and *Chaetomium* sp. These will need follow-up to determine their specific classification.

10.3 Succession in Coprophilous Ascomycetes

We observed the coprophilous ascomycetes as they sporulated and developed to maturity. The sporulation time was divided into 11 weekly periods starting from 0-7 and ending at 71-77 days. Ascomycetes that sporulated were assigned into the relevant age period (cohort). It was observed that succession started with *Ascobolus* followed by *Saccobolus*, *Schizothecium, Podospora, Arnium, Sordaria, and Zopfiella* and ended with *Zygopleurage*. Some taxa such as *Podospora communis*, *Saccobolus depauperatus* and *Iodophanus difformis* sporulated across the entire incubation period. It was also noted that even within the same genus there was a sequential sporulation pattern.

10.4 Significance and Publications Resulting from this Survey

During the course of this study, I visited four sampling sites at least twice and also got random opportunities to collect dung samples from Nairobi National Park with colleagues who were carrying out other biodiversity research in the park. In total I made about 12 field trips and collected 62 samples of dung. I have examined over 100 fungal specimens from which I concentrated on ten genera represented by 41 species. I made thorough examination of *Ascobolus*, *Saccobolus*, *Schizothecium, Podospora, Chaetomium*, *Arnium*, *Zygopleurage*, *Sordaria* and *Sporomiella* from wildlife dung.

I also encountered and examined specimens from other genera, such as, *Cercophora*, *Iodophanus*, *Coproptus*, *Lasiobolus* and *Microascus* which due to time constraints I could not make part of this thesis. These will be analyzed further for publication in future. From this morphological examination, I have described two new species from the genus *Ascobolus*, 17 new records and six undetermined species for future follow-up.

It is noteworthy that this is the first detailed inventory of coprophilous ascomycetes from wildlife dung by a Kenyan. The results of my work have been published in eight peer-reviewed papers, in which I am the first author. Each of these publications introduces eye-opener findings which have improved the understanding of coprophilous ascomycetes from wild herbivores. My work and the resultant publications form a sound baseline for future research in coprophilous ascomycetes from wildlife dung. The work has added significant knowledge and understanding of the taxonomy and ecological relationships between coprophilous ascomycetes and their animal hosts in Kenya. This work has also opened new frontiers of microbial conservation and management in Kenya. I, therefore, believe my contribution to the field of science, particularly in relation to mycology and specifically wildlife coprophilous ascomycetes is highly significant and thus qualifies for the award of a Master of Science degree in Biological Sciences.

10.5 List of Publications

Mungai, P. G., Chukeatirote, E., Njogu, J. G. & Hyde, K. D. (2012). Coprophilous ascomycetes in Kenya: *Chaetomium* species from wildlife dung. **Current Research in Environmental & Applied Mycology, 2**(2), 113–128.

Mungai, P. G., Njogu, J. G., Chukeatirote, E. & Hyde, K. D. (2012). Coprophilous ascomycetes in Kenya: *Sporormiella* from wildlife dung. **Mycology: An International Journal of Fungal Biology,** 3:4, 234-251.

Mungai, P. G., Chukeatirote, E., Njogu, J. G. & Hyde, K. D. (2012). Studies of coprophilous ascomycetes in Kenya. *Podospora* species from wildlife dung. **Mycosphere, 3**(6), 978–981.

Mungai, P. G., Njogu, J. G., Chukeatirote, E. & Hyde, K. D. (2012). Studies of Coprophilous ascomycetes in Kenya – *Schizothecium* from wildlife dung. **Current Research in Environmental & Applied Mycology, 2** (1), 84–97.

Mungai, P. G., Chukeatirote, E., Njogu, J. G. & Hyde, K. D. (2012). Studies of Coprophilous ascomycetes in Kenya: Sordariales from wildlife dung. **Mycosphere, 3**(4), 437–448.

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.

Mungai, P. G., Njogu, J. G., Chukeatirote, E. & Hyde, K. D. (2012). Studies of Coprophilous ascomycetes in Kenya – *Ascobolus* species from wildlife dung. **Current Research in Environmental & Applied Mycology, 2**(1), 1–16.

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10.6 Future Work

The importance of documenting Kenyan coprophilous ascomycetes cannot be overemphasized. Morphological examination complemented by phylogenetic studies will need to be carried out to resolve problematic groups such as *Sporormiella* and *Chaetomium*. Other groups of dung fungi such as myxomycetes, hyphomycetes, basidiomycetes and zygomycetes need to be included in future studies.

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APPENDICES

APPENDIX A

Detailed information on material Examined in this Study

Ascomycetes species	Date	Sample No.	Park/ Reserve	Veget. type	Longitude	Latitude	Alt. (m) asl	Animal species (dung type)
Podospora communis, *Sporormiella herculea*	18-8-09	KWSNNP001-2009	Nairobi	Savannah grassland	025273715	9849130	5629	Buffalo (*Syncerus caffer*)
Zygopleurage zygospora	18-8-09	KWSNNP002-2009	Nairobi	Savannah grassland	025273715	9849130	5629	Zebra (*Equus burchellii*)
Podospora communis	18-8-09	KWSNNP003-2009	Nairobi	Savannah grassland	0252737	9847748	5629	Impala (*Aepyceros melampus*)
Podospora anserina, *Podospora communis*	18-8-09	KWSNNP004-2009	Nairobi	Bushed grassland	0252737	9847748	5629	Rhino (*Diceros bicornis*)
Podospora communis	18-8-09	KWSNNP005-2009	Nairobi	Savannah	0253662	987088	5629	Giraffe (*Giraffa camelopardalis*)
Podospora communis	18-8-09	KWSNNP006-2009	Nairobi	grassland	0254976	9850544	5629	Hippo (*Hippopotamus amphibious*)
Saccobolus depauperatus, *Sporormiella leporina*, *Sporormiella* sp.,	26-8-09	KWSSH001-2009	Shimba Hills	Coastal tropical rainforest	S04°13’35.6”	E039°25’31.6”	373	Giraffe (*Giraffa camelopardalis*)
Saccobolus depauperatus, *Zygopleurage zygospora*	26-8-09	KWSSH003-2009	Shimba Hills	Open grassland	S04°14’35.4”	E039°26’07.1”	361	Hartebeest (*Alcelaphus buselaphus*)
Zygopleurage zygospora	26-8-09	KWSSH004B-2009	Shimba Hills	Open grassland	S04°14’35.6”	E039°26’07.1”	366	Buffalo (*Syncerus caffer*)
Zygopleurage zygospora, *Sporormiella minima*	26-8-09	KWSSH005B-2009	Shimba Hills	Open grassland	S04°14’22.4”	E039°26’23.2”	374	Impala (*Aepyceros melampus*)
Ascobolus amoenus, *Sporormiella minima*	27-8-09	KWSTE002B-2009	Tsavo East	Bushed grassland	S03°02’52.3”	E038°54’37.0”	354	Impala (*Aepyceros elampus*)

Ascomycetes species	Date	Sample No.	Park/ Reserve	Veget. type	Longitude	Latitude	Alt. (m) asl	Animal species (dung type)
Ascobolus amoenus, Ascobolus calesco, Zygopleurage zygospora, Sporormiella minima	27-8-09	KWSTE003B-2009	Tsavo East	Riverine	S03°02’52.3”	E038°54’37.0”	354	Elephant (*Loxodonta africana cyclotis*)
Podospora communis	27-8-09	KWSTE004B-2009	Tsavo East	Riverine	S03°02’29.7”	E038°41’35.8”	354	Zebra (*Equus burchellii*)
Ascobolus calesco, Saccobolus depauperatus, Podospora anserina, Zopfiella aff. *erostrata, Sporormiella minima, Chaetomium convolutum*	27-8-09	KWSTE005B-2009	Tsavo East	Riverine	S03°02’29.7”	E038°41’35.8”	354	Dikdik (*Madoqua kirki*)
Ascobolus amoenus, Ascobolus tsavoensis, Podospora communis	27-8-09	KWSTE006B-2009	Tsavo East	Riverine	S03°02’24.9”	E038°42’57.1”	343	Waterbuck (*Kobus ellipsiprymnus*)
Ascobolus amoenus, Ascobolus calesco, Podospora communis	27-8-09	KWSTE007B-2009	Tsavo East	Riverine	S03°04’26.9”	E038°48’32.3”	305	Buffalo (*Syncerus caffer*)
Ascobolus calesco, Zygopleurage zygospora	27-8-09	KWSTE008B-2009	Tsavo East	Riverine	S03°02’52.2”	E038°50’52.5”	236	Giraffe (*Giraffa camelopardalis*)
Podospora communis	29-8-09	KWSANP001-2009	Aberdares	Montane forest	S00°21’26.4”	E036°51’23.8”	2004	giant forest hog (*Hylochoerus meinertzhageni*)
	29-8-09	KWSANP002-2009	Aberdares	Montane forest	S00°21’26.4”	E036°51’23.8”	2004	Buffalo (*Syncerus caffer*)
Saccobolus depauperatus	29-8-09	KWSANP003-2009	Aberdares	Montane forest	S00°21’35.0”	E036°52’47.8”	2074	African elephant (*Loxodonta africana cyclotis*)
Ascobolus calesco, Podospora anserina, Podospora communis	29-8-09	KWSANP004-2009	Aberdares	Montane forest	S00°21’42.0”	E036°52’55.9”	2076	Black rhinoceros (*Diceros bicornis*)
Saccobolus diffusus, Saccobolus platensis, Podospora communis, Schizothecium dakotense, Zygopleurage zygospora, Sporormiella intermedia	29-8-09	KWSANP005-2009	Aberdares	Montane forest	S00°20’23.2”	E036°47’11.1”	2075	Waterbuck (*Kobus ellipsiprymnus*)
Podospora communis	30-8-09	KWSACC001-2009	Aberdares C.C. Sanctuary	Wooded grassland	S00°19’28.1”	E036°55’54.3”	2061	Impala (*Aepyceros melampus*)

Ascomycetes species	Date	Sample No.	Park/ Reserve	Veget. type	Longitude	Latitude	Alt. (m) asl	Animal species (dung type)
Saccobolus depauperatus, Podospora anserina, Podospora communis, Sporormiella aff. *minipascua, Sporormiella* aff. *muskokensis*	30-8-09	KWSACC003-2009	Aberdares C.C. Sanctuary	Bushed grassland	S00°19’28.1”	E036°55’54.3”	2161	Giraffe (*Giraffa camelopardalis*)
Chaetomium muelleri, Chaetomium seminis-citrulli, Chaetomium sp.	30-8-09	KWSACC004-2009	Aberdares C.C. Sanctuary	Forest /woodland	S00°19’25.4”	E036°55’50.7”	2025	Dikdik (*Madoqua kirki*)
Ascobolus amoenus	31-8-09	KWSNNP007-2009	Nairobi	Savanna	S01°21’21.8”	E036°46’51.8”	1765	Zebra *(Equus burchellii)*
Saccobolus depauperatus, Saccobolus truncatus, Schizothecium curvuloides var. *curvuloides, Schizothecium dakotense, Schizothecium dubium, Sporormiella intermedia, Sporormiella minima, Chaetomium globosum*	31-8-09	KWSNNP008-2010	Nairobi	Savanna	S01°21’15.1”	E036°46’54.1”	1768	Impala (*Aepyceros melampus*)
Saccobolus depauperatus, Chaetomium globosum	31-8-09	KWSNNP009-2009	Nairobi	Savanna	S01°21’12.2”	E036°47’18.5”	1752	black rhino (*Diceros bicornis*)
Schizothecium dakotense, Sporormiella aff. *minipascua*	31-8-09	KWSNNP010-2009	Nairobi	Savanna	S01°21’25.5”	E036°47’51.6”	1748	Giraffe (*Giraffa camelopardalis*)
Ascobolus calesco, Podospora anserina, Schizothecium curvuloides var. *curvuloides*	31-8-09	KWSNNP012-2009	Nairobi	Savanna grassland	S01°20’50.1”	E036°47’51.3”	1695	Hippopotamus *(Hippopotamus amphibious*)
Schizothecium curvuloides var. *curvuloides, Chaetomium convolutum, Chaetomium globosum*	31-8-09	KWSNNP013-2009	Nairobi	Savanna	S01°20’50.1”	E036°47’51.3”	1681	hartebeest (*Alcelaphus buselaphus*)
Ascobolus nairobiensis	20-8-10	KWSNNP014-2010	Nairobi	Grassland	37M 0255191	9849808	1693	Black rhino (*Diceros bicornis*)
Saccobolus citrinus, Podospora communis, Schizothecium conicum, Zygopleurage zygospora	20-8-10	KWSNNP015-2010	Nairobi	Grassland	37M0255191	9849808	1693	Buffalo (*Syncerus caffer*)

Ascomycetes species	Date	Sample No.	Park/ Reserve	Veget. type	Longitude	Latitude	Alt. (m) asl	Animal species (dung type)
Podospora argentinensis, Podospora australis	20-8-10	KWSNNP016-2010	Nairobi	Grassland	37M0255297	9848528	1693	Zebra (*Equus burchellii)*
Ascobolus bistisii, Saccobolus citrinus, Podospora australis, Arnium arizonense	20-8-10	KWSNNP017B-2010	Nairobi	Grassland	37M0257082	9850692	1668	Giraffe (*Giraffa camelopardalis*)
Ascobolus bistisii, Ascobolus immersus, Saccobolus infestans	20-8-10	KWSNNP018-2010	Nairobi	Grassland	37M0257082	9850692	1668	Zebra (*Equus burchellii*)
Ascobolus bistisii, Ascobolus immersus, Saccobolus citrinus, Saccobolus versicolor	20-8-10	KWSNNP020-2010	Nairobi	Grassland	37M0255729	9849626	1680	Impala (*Aepyceros melampus*)
Saccobolus citrinus	20-8-10	KWSNNP021-2010	Nairobi	Grassland	37M0254965	9850592	1685	Hippo (*Hippopotamus amphibious*)
Saccobolus truncatus, Sporormiella minima, Chaetomium convolutum	23-9-08	KWSTE002A-2008	Tsavo East	Riverine	S03°21.666	E038°38.772	514	Giraffe (*Giraffa camelopardalis*)
Zygopleurage zygospora	23-9-08	KWSTE003A-2008	Tsavo East	Riverine	S03°21.666	E038°38.772	514	Elephant (*Loxodonta africana cyclotis*)
Saccobolus depauperatus, Saccobolus platensis, Podospora anserina, Zopfiella longicaudata	23-9-08	KWSTE005A-2008	Tsavo East	Riverine	S03°21.666	E038°38.772	514	Elephant (*Loxodonta africana cyclotis*)
Sporormiella minima, Chaetomium convolutum	23-9-08	KWSTE006A-2008	Tsavo East	Riverine	S03°032.447	E038°37.828	514	Dikdik (*Madoqua kirki*)
Podospora anserina, Podospora communis, Sporormiella minima	23-9-08	KWSTE007A-2008	Tsavo East	Riverine	S03°21.064	E038°37.501	514	Zebra (*Equus burchellii*)
Saccobolus depauperatus, Sporormiella minima	23-9-08	KWSTE008A-2008	Tsavo East	Riverine	S03°21.064	E038°37.501	514	Buffalo (*Syncerus caffer*)
Schizothecium glutinans	24-9-08	KWSSH005A-2008	Shimba Hills	Riverine	S04°14’04.4”	E039°26’06.8”	361	Sable antelope (*Hippotragus niger*)
Saccobolus depauperatus, Sordaria fimicola, Sporormiella minima	19-4-09	KWSKIN004-2009	Kinondo	Riverine	S04°25.197	E039°32.602	18	Dikdik (*Madoqua kirki*)

Collected by: Paul Mungai

APPENDIX B

ABSTRACTS OF PUBLICATIONS AUTHORED

Current Research in Environmental & Applied Mycology Doi 10.5943/cream/1/2/2

Coprophilous ascomycetes of northern Thailand

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Mungai P, Hyde KD, Cai L, Njogu J, Chukeatirote K 2011 – Coprophilous ascomycetes of northern Thailand. Current Research in Environmental & Applied Mycology 1(2), 135–159, Doi 10.5943/cream/1/2/2

The distribution and occurrence of coprophilous ascomycetes on dung of Asiatic elephant, cattle, chicken, goat and water buffalo in Chiang Rai Province, northern Thailand was investigated between March and May, 2010. A moist chamber culture method was employed. Species from eleven genera in Sordariales, Pleosporales, Pezizales, Thelebolales and Microascales were identified. Some of the species examined are new records for Thailand. The most common species were *Saccobolus citrinus*, *Sporormiella minima*, *Ascobolus immersus* and *Cercophora kalimpongensis*. Most fungal species were found on cattle dung. Chicken dung, a rarely reported substrate for coprophilous fungi, had the least fungal species.

Key words – *Ascobolus* – *Cercophora* – dung types – moist chamber – *Saccobolus* – *Sporormiella* – substrate.

Current Research in Environmental & Applied Mycology Doi 10.5943/cream/2/1/4

Studies of coprophilous ascomycetes in Kenya: Coprophilous *Schizothecium* from wildlife dung

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Mungai PG, Njogu JG, Chukeatirote E, Hyde KD 2011 – Studies of coprophilous ascomycetes in Kenya. Taxonomy of coprophilous *Schizothecium* from wildlife dung. Current Research in Environmental & Applied Mycology 2(1), 84–97, Doi 10.5943/cream/2/1/4

Schizothecium encompasses species whose morphological features make them easily confused with *Podospora* and *Cercophora*. This study, carried out between September 2008 and October 2010, set out to characterize *Schizothecium* species from wildlife dung and determine their ecological attributes. Dung from Cape buffalo, zebra, giraffe, hippopotamus, impala, Jackson's hartebeest, sable antelope and waterbuck was incubated in a moist chamber culture. Morphological features of sporulating ascomycetes were used to characterize and identify the species. Five species, *Schizothecium conicum*, *S. curvuloides* var. *curvuloides*, *S. dakotense*, *S. dubium* and *S. glutinans* were isolated and described. *Schizothecium dakotense, S. dubium* and *S. glutinans* were new records. *Schizothecium curvuloides* var. *curvuloides* and *S. dakotense* were fairly common.

Key words – *Cercophora* – ecological attributes– moist chamber culture – *Podospora* – species diversity – wildlife

Current Research in Environmental & Applied Mycology Doi 10.5943/cream/2/1/1

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

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Mungai PG, Njogu JG, Chukeatirote E, Hyde KD 2012 – Studies of coprophilous ascomycetes in Kenya – *Ascobolus* species from wildlife dung. Current Research in Environmental & Applied Mycology 2(1), 1-16, Doi 10.5943/cream/2/1/1

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

Mycosphere Doi 10.5943/mycosphere/3/2/2/

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

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Mungai PG, Chukeatirote E, Njogu JG, Hyde KD 2012 – Coprophilous ascomycetes in Kenya: *Saccobolus* species from wildlife dung. Mycosphere 3(2), 111-129, Doi 10.5943/mycosphere/3/2/2/

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*

Mycosphere Doi 10.5943/mycosphere/3/4/7

Studies of coprophilous ascomycetes in Kenya: Sordariales from wildlife dung

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Mungai PG, Chukeatirote E, Njogu JG, Hyde KD 2012 – Studies of coprophilous ascomycetes in Kenya: Sordariales from wildlife dung. Mycosphere 3(4), 437–448, Doi 10.5943 /mycosphere/3/4/7

In our continuing series on coprophilous fungi from wild herbivores moist chamber dung cultures from African elephant, Cape buffalo, dikdik, giraffe, impala, Jackson's hartebeest, waterbuck and zebra found in Kenyan National Parks and Reserves were examined for sporulating coprophilous Sordariales. *Arnium arizonense*, *Sordaria fimicola* and *Zopfiella longicaudata* are reported for the first time in Kenya while *Zygopleurage zygospora* is a very frequent species on wildlife dung. *Zopfiella* affinis *erostrata* awaits further examination as it could be a novel species.

Key words – *Arnium* – national parks – *Sordaria* – wild herbivores – *Zopfiella* – *Zygopleurage*

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Studies of coprophilous ascomycetes in Kenya. *Podospora* species from wildlife dung

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Moist chamber cultures were made from wildlife dung obtained from national parks in Kenya. Ten dung types produced 28 specimens of *Podospora*. Five species, *Podospora anserina*, *P. argentinensis*, *P. australis*, *P. communis* and *P. minor* are described using their morphological features. *Podospora minor* seems to be a rare species and is recorded for the first time in Kenya. *Podospora communis, P. anserina* and *P. australis* are the most common species on dung types examined.

Key words – *Arnium* – biodiversity – conservation – perithecioid – *Schizothecium* – taxonomy

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Coprophilous ascomycetes in Kenya: *Sporormiella* from wildlife dung

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Fresh specimens of *Sporormiella* obtained from the dung of African elephant, Cape buffalo, dikdik, giraffe, impala and waterbuck and incubated in a moist chamber were examined. *Sporormiella herculea*, *S. intermedia*, *S. leporina*, *S. minima*, *S.* aff. *minipascua*, *S.* aff. *muskokensis*, *Sporormiella* sp. and *Sporormiella* aff. *teretispora* were observed and described. A new record *Sporormiella leporina* and three unidentified species *S.* aff. *minipascua*, *Sporormiella* sp. and *Sporormiella* aff. *teretispora* are illustrated. *Sporormiella minima*, *S. intermedia* and *S.* aff. *minipascua* are the most common on wildlife dung examined.

Keywords: conservation areas; ecology; national parks; pseudothecia; Sporormiaceae; taxonomy; wild herbivore

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Coprophilous ascomycetes in Kenya: *Chaetomium* species from wildlife dung

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In our studies to document the diversity and distribution of coprophilous ascomycetes in Kenya, we collected several species in *Chaetomium*. This genus, comprises a large group of saprobic ascomycetes growing on dung and other cellulose-rich substrates. In the present study we collected wild animal dung from different ecosystems in Kenya. The dung substrate was laboratory cultured using a moist chamber method. Five taxa, *Chaetomium convolutum*, *C. globosum*, *C. muelleri, C. seminis-citrulli* a new record and *Chaetomium* sp., probably a novel species, are examined, described and illustrated. *Chaetomium convolutum* and *C. globosum* are the most common taxa in the dung studied. *Chaetomium* seems to have a preference of growing on browser animal dung.

Key words – basal fascicles – biodiversity – Chaetomiaceae – saprobic – taxonomy – wild animals

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