

Morphological diversity of saprophytic coprophilous fungi from selected domestic and wild herbivore dung in different terrestrial ecosystems of KwaZulu-Natal Province, South Africa

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ABSTRACT

Coprophilous fungi are specialised saprotrophs adapted to colonise herbivore dung, where they contribute to decomposition, nutrient cycling, and spore-mediated dispersal through dung vegetation-herbivore pathways. Despite their ecological importance, baseline records of dung-inhabiting fungi remain limited for many African regions, including KwaZulu-Natal, South Africa. This study provides a morphology-based baseline survey of coprophilous fungi associated with domestic and wild herbivore dung collected from Ukulinga Research Farm, Tala Private Game Reserve, and Phinda Private Game Reserve. Fresh dung samples were incubated under moist-chamber conditions for up to 25 days, and developing fruiting bodies were examined using stereomicroscopy and light microscopy. The study focused on the genera *Ascobolus* and *Pilobolus* because these were the most consistently observed and morphologically diagnosable taxa during incubation. Across 27 dung-derived incubation samples, morphotypes consistent with *Pilobolus* and *Ascobolus* were recorded, with *Pilobolus* generally appearing earlier during incubation and *Ascobolus* appearing later. Diagnostic characters included trophocysts, phototropic sporangiophores, subsporangial vesicles, and pigmented sporangia in *Pilobolus*, and apothecoid ascomata, operculate asci, pigmented ascospores, and gelatinous sheaths in *Ascobolus*. Because molecular sequence data were not generated, species-level identifications are treated conservatively, and uncertain collections are reported as morphotypes or *cf.* assignments. This study establishes a specimen-linked morphological baseline for selected coprophilous fungi in KwaZulu-Natal and provides a framework for future integrative taxonomic studies using voucher material, raw morphometric data, and molecular phylogenetic confirmation.

1. Introduction

Coprophilous fungi (copro: dung; philous: loving) are dung-associated species adapted to colonize, fruit, and disperse from animal faeces (Kaur et al., 2019; Ojwach, 2018). Herbivore dung is a heterogeneous substrate comprising partially digested vegetation, gut microbiota, and additional material such as soil particles, hair, and mineral sediments (Holter, 2016; Shahack-Gross, 2011). The composition of dung is largely determined by the diet of the host animal, which includes

dicot leaves, seeds, fruits, bark, and pollen, thus creating a range of microhabitats with varying physicochemical properties and nutrient profiles (Portillo et al., 2012; Shahack-Gross, 2011). These conditions support diverse microbial communities, including coprophilous fungi that play a key ecological role in the decomposition of dung. Through their metabolic activity, these fungi accelerate the breakdown of organic matter, facilitating nutrient cycling and contributing to soil fertility and ecosystem functioning (Aluoch et al., 2017; Sarrocco, 2016).

Coprophilous fungi display a variety of life-history strategies that

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enable them to persist in these transient habitats (Thomas and Thomas, 2022). Obligate coprophilous species typically rely on passage through the digestive tract of herbivores to break spore dormancy and enable germination, whereas facultative species can colonize dung without requiring gut passage (Dix, 2012; Richardson, 2001). Several physiological and ecological adaptations promote survival and reproduction in dung habitats, including tolerance to alkaline pH and high moisture, and strong dispersal mechanisms that reintroduce spores onto vegetation to be re-ingested (Kuthubutheen and Webster, 1986; Bell, 1983; Abdullah, 1982). Fungal colonization of dung typically follows a well-defined successional sequence. Early colonizers are often zygomycetous fungi, particularly members of the order Mucorales, which rapidly exploit freshly deposited dung (Stephenson, 2010). These are followed by apothecial and perithecial Ascomycota, and eventually by Basidiomycota that produce more conspicuous fruiting bodies during later stages of decomposition (Richardson, 2001; Weber and Webster, 1998; Gnanam, 2019; Khandare, 2021; Stephenson, 2022). Among coprophilous taxa, *Pilobolus* (Mucorales) is notable for its ballistic spore dispersal mechanism and its documented role in dispersing lungworm larvae (*Dictyocaulus* spp.), which can contribute to parasitic bronchitis in grazing herbivores (Dima and Fikedu). *Ascobolus* (Pezizales) comprises apothecioid Ascomycota with pigmented, thick-walled ascospores and distinctive ascus features that support morphological species delimitation (Bell, 1983; van Brummelen, 1967). Studies of coprophilous fungi in African wildlife systems have revealed considerable taxonomic diversity, with genera such as *Ascobolus* and *Pilobolus* being among the most frequently reported due to their ecological roles in dung decomposition and nutrient cycling (Njouonkou et al., 2025; Khairalla, 2025; Morandi, 2025; Charria-Girón et al., 2025; Malygina et al., 2025). However, comprehensive baseline surveys remain limited for many regions, including large areas of South Africa. Existing studies have documented diverse coprophilous assemblages in wildlife dung, yet the distribution, morphological variation, and ecological roles of these fungi remain incompletely understood (Ndozi et al., 2025; Aluoch et al., 2015; Krug et al., 2004; Ebersohn and Eicker, 1992).

Coprophilous fungi comprise a diverse assemblage of taxa belonging to several fungal groups, including representatives of the Ascomycota, Mucoromycota and Basidiomycota. Common coprophilous genera include *Ascobolus*, *Pilobolus*, *Sordaria*, *Podospira*, *Coprinopsis*, *Thelebolus* and *Preussia*, among many others. Although coprophilous fungi have been widely reported across southern Africa, targeted surveys of the genera *Pilobolus* and *Ascobolus* from herbivore dung in KwaZulu-Natal remain limited. This lack of focused studies has resulted in an important gap in baseline data necessary for regional biodiversity assessments and long-term ecological monitoring. Given the ecological significance of dung-associated fungi in nutrient cycling and decomposition, documenting their diversity is essential for improving our understanding of fungal community structure and ecosystem functioning in South African landscapes.

The primary aim of this study was to characterize the morphological diversity of coprophilous fungi associated with herbivore dung in the province of KwaZulu-Natal, South Africa, with particular emphasis on the genera *Ascobolus* and *Pilobolus*. Using established morphological identification keys by Richardson and Watling (2018) and Melo et al. (2020), this study aimed to characterise the morphological diversity of *Ascobolus* and *Pilobolus* associated with dung from domestic and wild herbivores across different terrestrial ecosystems in KwaZulu-Natal Province, South Africa. Specifically, we documented their occurrence, described diagnostic morphological and morphometric characteristics, and established a baseline dataset to support future biodiversity monitoring and integrative taxonomic studies incorporating molecular approaches.

This study was designed as an exploratory morphology-based baseline survey rather than a hypothesis-testing ecological experiment or molecular taxonomic revision. The genera *Ascobolus* and *Pilobolus* were selected because they were repeatedly observed during moist-chamber

incubation, possess distinctive diagnostic structures, and represent ecologically important components of dung fungal succession. We therefore aimed to: (i) document the occurrence of *Ascobolus* and *Pilobolus* morphospecies across selected domestic and wild herbivore dung substrates in KwaZulu-Natal; (ii) describe diagnostic morphological and morphometric characters linked to sample codes and voucher material; and (iii) provide a reproducible baseline dataset to support future molecular, phylogenetic, and ecological investigations of coprophilous fungi in southern African ecosystems.

2. Materials and methods

2.1. Study area and dung sampling

Fresh dung samples were collected aseptically into sterile bags, labelled with the host species, collection site, and date, and transported to the laboratory for incubation and analysis. Sampling followed a stratified, hindgut fermenter-focused design, in which herbivore species and study sites were treated as strata to ensure representative coverage across landscapes. Emphasis was placed on hindgut-fermenting herbivores (e.g., *Equus quagga*), due to their known influence on dung physicochemical properties and associated fungal communities. Within each stratum, freshly deposited dung samples were selected at random to minimize sampling bias. A total of twenty-seven samples were collected from both domestic herbivores (e.g., *Bos taurus*, *Capra hircus*) and wild herbivores (e.g., *Equus quagga*, *Aepyceros melampus*) across the three study sites (Ukulinga, Tala, and Phinda). Sampling was conducted across a range of terrestrial habitats in and around the province of KwaZulu-Natal, South Africa (Fig. 1A). The study included dung from herbivorous hosts, domesticated herbivores, wild herbivores, and large-bodied megaherbivores to capture a broad diversity of coprophilous fungal communities associated with different animal diets and ecological niches. Sampling sites were selected based on herbivore population density and feeding behaviour, as herbivores differ in their dietary strategies, with some species primarily grazers while others browse on leaves, shrubs, and woody vegetation. Sampling was conducted at three main locations in KwaZulu-Natal, South Africa. At Ukulinga Research Farm (Pietermaritzburg; 29.6627°S, 30.4050°E; approximately 400 ha), dung samples were collected from domesticated herbivores including *Bos taurus*, *Capra hircus*, and *Equus ferus caballus*. Additional samples were obtained from Tala Private Game Reserve (29.8257°S, 30.5416°E; approximately 3000 ha), where dung from wild herbivores such as *Ceratotherium simum*, *Hippopotamus amphibius*, *Giraffa camelopardalis*, *Kobus ellipsiprymnus*, *Tragelaphus scriptus*, *Aepyceros melampus*, and *Equus quagga* were collected. Further sampling was conducted at Phinda Private Game Reserve (27.8476°S, 32.3335°E; approximately 170 km²), where dung from wild herbivores, including *Ceratotherium simum* (white rhinoceros), *Tragelaphus angasi* (Inyala), *Syncerus caffer* (African buffalo), and other species, was collected. These locations were selected to capture variation in herbivore diet and habitat, as differences between grazing and browsing animals influenced dung physicochemical properties and plant residue composition, which in turn shape the diversity and structure of coprophilous fungal communities.

2.2. Moist-chamber incubation and microscopy

Herbivore dung was incubated using a moist-chamber method adapted from standard coprophilous fungal surveys of Aluoch et al., (2015). Dung portions were placed in labelled 100 mm x 15 mm Petri dishes lined with absorbent paper and moistened with sterile distilled water. Dishes were incubated under ambient laboratory conditions near windows to allow natural light/dark cycles for up to 25 days, with daily inspection for fruiting bodies. Fruiting bodies were sampled using sterile needles, mounted in water (or appropriate mounting media when necessary), and examined under a compound light microscope (Nikon Eclipse series equipped with a Nikon DS-Fi1 camera at magnifications of

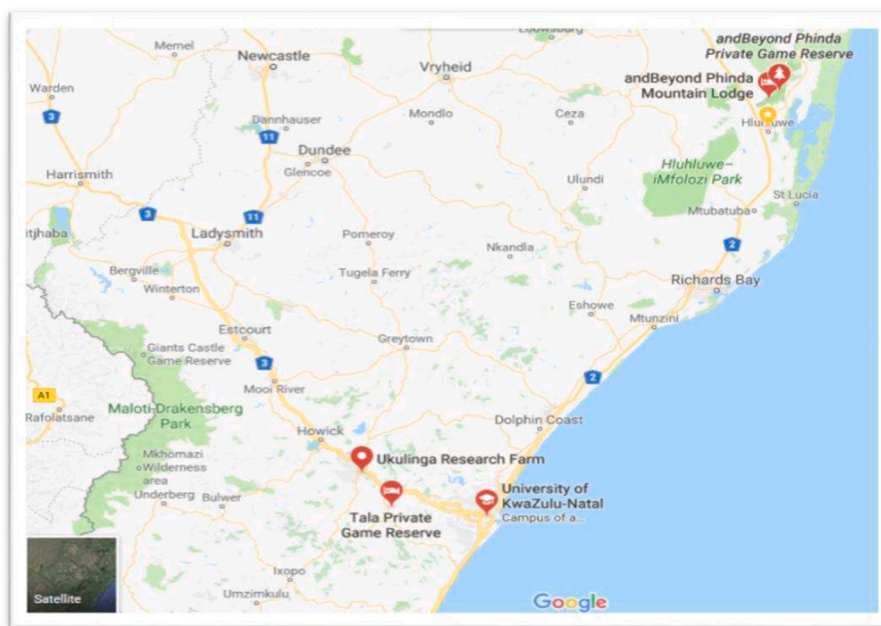


Fig. 1A. Map of KwaZulu-Natal Province, South Africa, showing the locations where herbivore dung samples were collected for this study. Sampling was conducted at Ukulinga Research Farm (Pietermaritzburg), Tala Private Game Reserve, and Phinda Private Game Reserve. These sites represent agricultural and protected wildlife ecosystems, where dung from domestic and wild herbivores was collected for the isolation and morphological characterization of the coprophilous fungal genera *Ascobolus* and *Pilobolus*.

Source: Base map adapted from Google Maps (<https://maps.google.com>), accessed on 22 July 2019.

X100 and X1000). Oil immersion (X1000) was used where required to enhance the resolution of fine morphological features. Morphometric measurements were made using calibrated imaging software (NIS Elements D v4.60), including sporangium/sporangiospore dimensions, trophocyst dimensions, ascus size, and other measurements. Where possible, 10 spores per structure were measured to estimate size ranges (following standard practice) (Aluoch et al., 2015).

2.3. Morphological identification

Taxonomic assignment was based primarily on morphological characteristics using established identification keys and species descriptions, including those of van Brummelen (1967), Bell (1983), and Doveri (2016), with additional reference to more taxonomic treatments (Viriato, 2008). These identifications were further supported by comparative morphological frameworks derived from studies of African coprophilous fungi (Aluoch et al., 2015). Diagnostic features such as sporangial structure, sporangiospore morphology, spore size and ornamentation, and reproductive structures were examined microscopically to facilitate species-level identification. However, morphological overlaps are well documented within certain genera, particularly *Pilobolus*, where cryptic diversity may occur. Consequently, where definitive species-level resolution could not be confidently achieved in the absence of molecular data, taxa were conservatively reported as morphospecies (Aluoch et al., 2017).

2.4. Voucher preservation, morphometric data, and reproducibility

Representative fruiting structures of *Ascobolus* and *Pilobolus* observed during moist-chamber incubation were preserved where sufficient material was available. Preserved material was linked to the original dung-derived isolate code, host dung source, sampling site, and morphological assignment. Voucher material is deposited at the University of KwaZulu-Natal under accession numbers AJ289046.1 and AB046383.1 and was placed within the same clade. Where voucher preservation was not possible because of limited or immature material,

this is indicated in the specimen metadata table. Morphometric measurements were recorded from mature diagnostic structures whenever possible. These included trophocysts, sporangiospores, subsporangial vesicles, sporangia, sporangiospores, ascomata, asci, and ascospores. Measurements were linked directly to isolate codes and voucher numbers. Raw measurement data are provided in **Supplementary Table S1**. Taxa lacking mature diagnostic structures were not assigned definitive species names and are reported as genus-level morphotypes or cf. taxa.

2.5. Data analysis

Observed focal taxon richness was calculated as the number of morphologically distinguishable *Ascobolus* and *Pilobolus* morphospecies recorded in each dung-derived incubation sample. Richness values were subsequently summarised by herbivore host and sampling site. Because sampling effort and replication differed among host species and locations, the analyses were descriptive, and no inferential comparisons were performed. Shannon and Simpson diversity indices were not calculated because quantitative abundance data for individual morphospecies were not collected consistently across all incubation samples. Accordingly, the reported values represent observed focal richness rather than total fungal diversity.

3. Results

3.1. Dung sources and isolate metadata

A total of 27 dung-derived incubation samples were examined from Ukulinga Research Farm, Tala Private Game Reserve, and Phinda Private Game Reserve (Table 1; Fig. 1A). The study focused specifically on morphologically distinguishable taxa assigned to *Ascobolus* and *Pilobolus*. Therefore, the richness values reported here refer only to the focal *Ascobolus*-*Pilobolus* morphospecies and do not represent total coprophilous fungal richness across all fungal groups present in the dung samples. Across the 27 dung-derived samples, observed focal taxon richness

Table 1

Species richness of coprophilous fungi across herbivore dung types and sampling sites in KwaZulu-Natal, South Africa.

Isolate number	Herbivore dung species	Assigned code	Sampling site	Number of dung piles	Number of species identified
1	Black Rhino (<i>Diceros bicornis</i>) dung	XROT- 1	Tala Game Reserve	9	2
2	White rhino (<i>Ceratotherium simum</i>) dung	XWRP- 2	Phinda Game Reserve	14	4
3	Black Rhino dung	XROT- 3	Tala Game Reserve	8	4
4	Black Rhino dung	XROT- 4	Tala Game Reserve	5	2
5	Black Rhino dung	XROT- 5	Tala Game Reserve	3	4
6	Black Rhino dung	XROT- 6	Tala Game Reserve	9	4
7	Black Rhino dung	XROT- 7	Tala Game Reserve	9	2
8	Black Rhino dung	XROT- 8	Tala Game Reserve	7	2
9	Goat (<i>Capra hircus</i>) dung	XGOU- 9	Ukulinga Farm	23	1
10	Goat dung	XGOU- 10	Ukulinga Farm	17	1
11	Goat dung	XGOU- 11	Ukulinga Farm	20	1
12	Giraffe (<i>Giraffa giraffa</i>) dung	XGFT-12	Tala Game Reserve	23	2
13	Inyala (<i>Tragelaphus angasii</i>) dung	XIOP- 13	Phinda Game Reserve	15	2
14	Giraffe dung	XGFT- 14	Tala Game Reserve	9	1
15	Zebra dung	XZBT- 15	Tala Game Reserve	13	1
16	Giraffe dung	XGFT- 16	Tala Game Reserve	14	2
17	Waterbuck (<i>K. ellipsiprymnus</i>) dung	XWBT- 17	Tala Game Reserve	12	4
18	Waterbuck dung	XWBT- 18	Tala Game Reserve	16	2
19	Waterbuck dung	XWBT- 19	Tala Game Reserve	19	2
20	Bushbuck (<i>Tragelaphus sylvaticus</i>) dung	XBBT- 20	Tala Game Reserve	8	2
21	Buffalo (<i>Syncerus caffer</i>) dung	XOBP- 21	Phinda Game Reserve	23	5
22	Impala dung	XIMT- 22	Tala Game Reserve	18	1
23	Impala (<i>Aepyceros melampus</i>) dung	XIMT- 23	Tala Game Reserve	23	1
24	Impala dung	XIMT- 24	Tala Game Reserve	14	1
25	Impala dung	XIMT- 25	Tala Game Reserve	12	2
26	Bovine (<i>Bos taurus/indicus</i>) dung	XOCU- 26	Ukulinga Farm	11	3
27	Bovine dung	XOCU- 27	Ukulinga Farm	21	3

ranged from one to five morphospecies per sample, with an overall mean of approximately 2.26 morphospecies per sample. Buffalo dung sample XOBP-21 from Phinda Private Game Reserve showed the highest observed richness, with five focal morphotypes. White rhinoceros dung sample XWRP-2 and several rhinoceros and waterbuck samples supported four focal morphospecies. Goat dung samples from Ukulinga Research Farm consistently yielded one focal morphotype. At the host level, buffalo dung showed the highest observed mean focal richness, followed by white rhinoceros, bovine, black rhinoceros, and waterbuck dung (Fig. 1B). At the site level, Phinda Private Game Reserve showed the highest observed mean focal richness, followed by Tala Private Game Reserve and Ukulinga Research Farm. These values are descriptive and reflect observed richness under the incubation conditions used in this study. They are not interpreted as statistically significant site- or host-level differences because sampling was uneven among dung types and locations. To facilitate comparison of fungal richness among herbivore hosts, the observed mean richness of *Ascobolus* and *Pilobolus* morphospecies is summarised in Fig. 1B. Buffalo dung exhibited the highest observed morphospecies richness, whereas goat dung consistently supported the lowest richness. These values represent descriptive summaries of the focal taxa recovered during moist-chamber incubation and should not be interpreted as statistically significant differences because sampling effort differed among herbivore hosts.

3.1.1. Observed focal richness among herbivore hosts

Observed richness of the focal *Ascobolus* and *Pilobolus* morphospecies varied among herbivore dung samples (Table 2). Buffalo dung sample XOBP-21 exhibited the highest observed richness, supporting five focal morphospecies. White rhinoceros dung and several black rhinoceros and waterbuck samples supported up to four morphospecies. In contrast, goat dung supported only one focal morphospecies. At the host level, buffalo dung showed the highest observed focal richness, followed by white rhinoceros, bovine, black rhinoceros and waterbuck dung. These patterns are presented descriptively because sampling effort and replication were uneven among herbivore hosts. They should therefore not be interpreted as statistically significant differences in fungal diversity.

Table 2

Descriptive summary of observed focal coprophilous fungal richness among herbivore dung hosts in KwaZulu-Natal, South Africa.

Herbivore host	Number of samples	Minimum richness	Maximum richness	Mean richness
Black rhinoceros (<i>Diceros bicornis</i>)	7	2	4	2.86
White rhinoceros (<i>Ceratotherium simum</i>)	1	4	4	4.00
Goat (<i>Capra hircus</i>)	3	1	1	1.00
Buffalo (<i>Syncerus caffer</i>)	1	5	5	5.00
Waterbuck (<i>Kobus ellipsiprymnus</i>)	3	2	4	2.67
Bovine (<i>Bos taurus/ indicus</i>)	2	3	3	3.00
Other herbivore hosts†	10	1	2	1.50

3.2. Fruiting dynamics and successional pattern

Fruiting bodies were observed within 3–10 days of moist-chamber incubation following methods described by Richardson and Watling (1969), Richardson and Watling (2018) and Bell (2005). Morphospecies consistent with *Pilobolus* generally appeared during the early incubation period and were recognised by the rapid development of sporangiophores and terminal sporangia. Apothecoid morphospecies consistent with *Ascobolus* generally appeared later, when mature ascospores became visible. This temporal sequence is consistent with the classical succession of coprophilous fungi on herbivore dung, in which fast-growing members of the Mucorales colonise fresh dung during the early stages of decomposition, followed by apothecial Ascomycota as decomposition progresses (Bell, 2005; Richardson and Watling, 2018). The timing and density of fruiting varied among dung substrates. However, because incubation samples were not replicated equally across host species and sites, these observations are presented as descriptive patterns rather than statistically tested ecological differences.

3.3. Morphology of *Pilobolus* morphospecies

Specimens assigned to *Pilobolus* were recognised by a combination of diagnostic characters, including a substrate-embedded trophocyst, rhizoidal anchorage, an unbranched phototropic sporangiophore, an inflated subsporangial vesicle, and a terminal dark sporangium. These features are consistent with generic descriptions of *Pilobolus* in established coprophilous fungal keys. *Pilobolus* morphospecies were readily recognized by a suite of characteristic morphological features typical of the genus (Fig. 2). These included: (i) a trophocyst semi-immersed within the dung substrate and anchored by rhizoidal extensions; (Fig. 3); (ii) a phototropic, unbranched sporangiophore; (iii) a terminal subsporangial vesicle supporting a dark, hemispherical sporangium; and (iv) ellipsoid sporangiospores. These diagnostic characters are consistent with descriptions used in previous surveys of African dung fungi (Aluoch et al., 2015) and align with morphometric ranges reported for *Pilobolus* taxa from similar environments (Aluoch et al., 2017). The sporangiophore consisted of a transparent, erect stalk that terminated in a swollen subsporangial vesicle bearing a darkly pigmented sporangium (Fig. 8). Observed specimens displayed the typical phototropic growth pattern associated with *Pilobolus*, orienting toward light sources to optimize spore dispersal. Morphometric analysis of key diagnostic structures, including sporangiophore length, subsporangial vesicle diameter, and sporangial size, revealed measurable variation among the observed morphotypes. Sporangiophore lengths ranged from approximately 300–400 μm , while the subsporangial vesicles measured between 700–900 μm in diameter. The sporangia were typically dark, globose to hemispherical, with diameters ranging from 150–200 μm . Sporangiospores were ellipsoid and enclosed within the sporangium until discharge. These morphological characteristics correspond closely with previously reported descriptions of *Pilobolus* species associated with herbivore dung (Subrahmanyam and Sambamurty, 2023). Because morphological traits alone may not fully resolve cryptic diversity within the genus *Pilobolus*, the observed forms are reported here as genus-level morphospecies unless species-level diagnostic features were clearly distinguishable. Nevertheless, the repeated occurrence of *Pilobolus* across multiple dung types suggests that this genus represents a common early coloniser of herbivore dung in the study area. Its well-known ballistic spore-discharge mechanism likely enhances dispersal efficiency, allowing spores to be projected away from the dung substrate onto surrounding vegetation, where they may subsequently be ingested by grazing animals, thereby completing the coprophilous life cycle. Because species-level delimitation in *Pilobolus* can be difficult using morphology alone, and because molecular sequence data were not available for this study, most collections are treated conservatively as

Pilobolus morphospecies or *Pilobolus* cf. taxa. Definitive species-level names are avoided unless the observed diagnostic structures correspond closely with published descriptions, and mature structures are clearly visible.

3.4. Morphology of *Ascobolus* morphospecies

Members of the genus *Ascobolus* were observed as small apothecioid fruiting bodies developing on the surface of incubated dung samples. These structures appeared as cupulate to discoid ascomata that were superficial to semi-immersed in the dung substrate, consistent with classical morphological descriptions of coprophilous *Ascobolus* species (Bell, 1983; van Brummelen, 1967). The apothecia were typically solitary to gregarious and displayed distinct pigmentation patterns ranging from yellowish green to olive or brownish tones. These fruiting bodies generally appeared later in the incubation period than *Pilobolus*, reflecting the established successional dynamics of coprophilous fungal communities (Richardson, 2001). Diagnostic microscopic features included operculate asci containing eight pigmented, thick-walled ascospores, frequently surrounded by a gelatinous sheath. These characteristics correspond closely with the defining morphological features of *Ascobolus* described in global taxonomic monographs and practical identification keys used in coprophilous studies (Aluoch et al., 2015; Bell, 1983; van Brummelen, 1967). Morphometric measurements revealed variation in ascospore size, shape, and pigmentation among the observed specimens, suggesting the presence of multiple morphotypes within the sampled dung communities.

Initial observations of dung samples revealed superficial to semi-immersed rounded fruiting bodies associated with dense hyphal colonization and dispersed spores (Fig. 4G–I). These structures were circular to subglobose in surface view, with pale hyaline margins and darker central contents scattered across the dung substrate. Although the overall morphology was consistent with a coprophilous ascomycete of the genus *Ascobolus*, mature asci, paraphyses, and fully resolved ascospores were not clearly visible in the preparation. Consequently, this material is retained as *Ascobolus* sp. pending further observation of mature diagnostic structures. More developed *Ascobolus* fruiting bodies were observed in zebra and white rhinoceros dung samples, where embedded reproductive structures and ascus-like formations were evident (Fig. 5A). In these samples, *Ascobolus amoenus* Oudem. was identified based on distinctive morphological characteristics. Ascomata were apothecioid, gregarious or scattered, and semi-immersed in the dung substrate, measuring approximately 500–700 μm in diameter. The receptacle was globose, becoming cupulate with age, with a yellowish-green to olive hymenial disc. The hypothecium consisted of globose

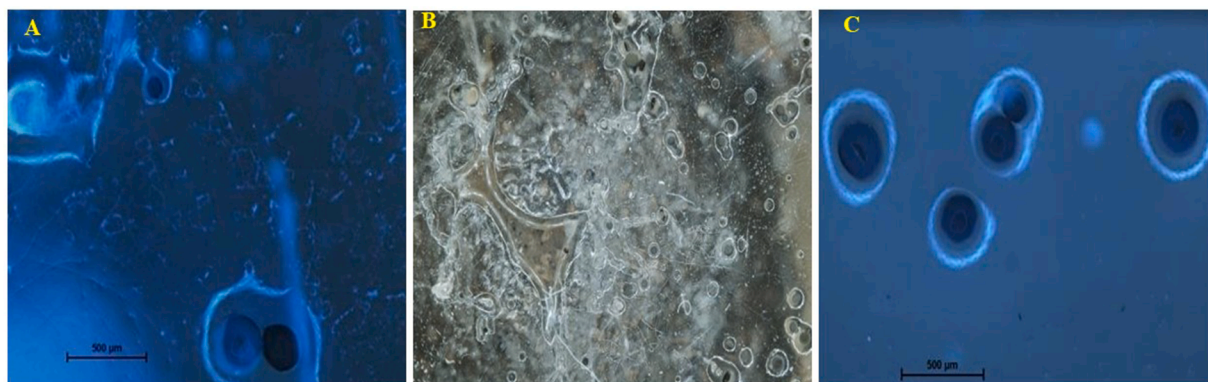


Fig. 2. *Pilobolus crystallinus* (F.H. Wigg.) Tode; (B) Bushbuck dung showing early colonization, developing sporangiophores and embedded reproductive structures. (A) Trophocyst subglobose to ovoid, 300–400 μm in diameter, embedded within dung substrate with rhizoidal extensions. (C) Sporangiophore erect, cylindrical, unbranched, phototropic, reaching 3–5 mm in height. The sub-sporangial vesicle is inflated and elliptical with smooth walls and faint orange pigmentation. Sporangium hemispherical to globose, dark brown to black at maturity, 400–500 μm in diameter. Columella dome-shaped, smooth-walled. Sporangiospores numerous, ellipsoidal to oval, smooth-walled, pale yellow to light brown, 7–10 $\times$ 4–6 μm .

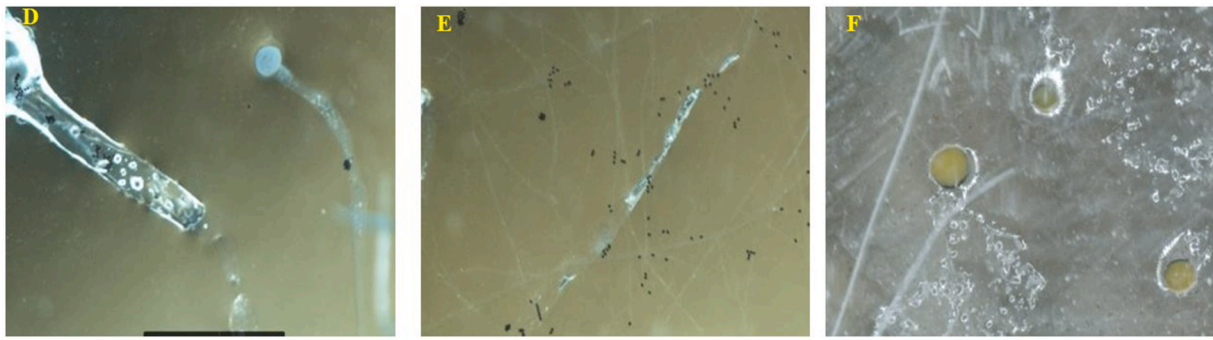


Fig. 3. *Pilobolus pullus* Massee (D); Cow dung showing elongated sporangiophores and terminal reproductive bodies typical of zygomycetous fungi. (E) Trophocysts globose to ovoid, hyaline, approximately 150–200 μm in diameter. Sporangia are erect, cylindrical, smooth-walled, measuring approximately 700–900 μm in length. (F) Subsporangial vesicle ovoid and hyaline with slight pigmentation. Sporangium hemispherical, dark brown to black, 250–300 μm in diameter. Columella is dome-shaped and smooth-walled. Zygospores are oval to cylindrical, yellowish, with homogenous internal contents. Observed in cow dung samples collected from Ukulinga Research Farm. The relatively small trophocysts and smaller sporangial sizes distinguish this species from *P. crystallinus*.

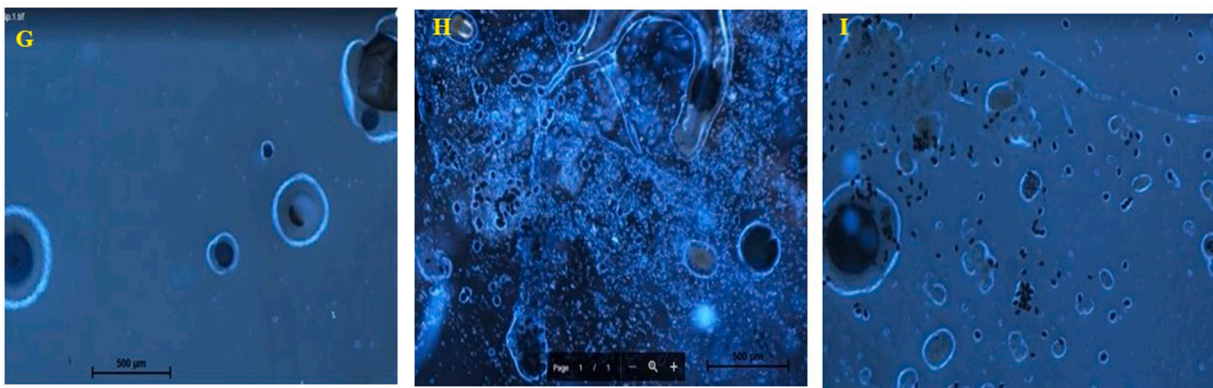


Fig. 4. *Ascobolus* sp. aff. (G): Giraffe dung showing rounded fruiting structures, dense hyphal colonization and abundant dispersed spores. (H) Ascomata is superficial to semi-immersed, solitary to gregarious, circular to subglobose in surface view, with pale hyaline margins and dark central contents, scattered across the dung substrate. (I) Surrounding mycelium is sparse to moderate. The morphology is consistent with a coprophilous ascomycete of the genus *Ascobolus*. However, mature asci, paraphyses, and fully resolved ascospores were not clearly visible in the present preparation; therefore, the material is retained as *Ascobolus* sp. pending further observation of mature diagnostic structures.

hyaline cells, while the medullary excipulum displayed a textura globulosa arrangement. The ectal excipulum comprised pigmented angular cells forming a protective outer layer. Paraphyses were cylindrical, hyaline, septate, and embedded in a mucilaginous matrix. Asci were narrowly clavate, 8-spored, measuring approximately $180\text{--}250 \times 45\text{--}60 \mu\text{m}$. Ascospores were ellipsoidal, smooth-walled, and measured approximately $30\text{--}40 \times 20\text{--}25 \mu\text{m}$, becoming violet-brown at maturity. This species was recorded from zebra and giraffe dung collected at Tala Game Reserve and can be distinguished from related taxa by its relatively smaller ascospore size and characteristic greenish hymenial pigmentation.

Another species, *Ascobolus immersus* Pers., was identified from moist-chamber cultures of white rhinoceros and waterbuck dung collected from Phinda Game Reserve (Fig. 5B). Ascomata were solitary or gregarious, superficial to semi-immersed in the dung substrate, and sessile, measuring approximately 600–1000 μm in diameter. The receptacle was initially closed but became irregularly open with maturity, displaying yellowish-brown to olive-brown pigmentation. The hymenial disc was flat to convex with numerous protruding asci visible under stereomicroscopy. Paraphyses were filiform, hyaline, septate, and embedded in a mucilaginous matrix. Asci were broadly clavate to saciform, unitunicate, and operculate, measuring approximately $450\text{--}650 \times 90\text{--}110 \mu\text{m}$ and containing eight spores. The ascospores were ellipsoidal to subcylindrical, biserial, thick-walled, and smooth, measuring approximately $50\text{--}60 \times 25\text{--}35 \mu\text{m}$. At maturity, spores were violet to

dark purple and surrounded by a thin gelatinous sheath, a key diagnostic feature of *A. immersus*. This species is widely reported from herbivore dung ecosystems and is considered one of the most common coprophilous fungi associated with large grazing mammals.

Additional coprophilous morphotypes were observed in impala, horse, and goat dung samples, where relatively sparse fungal colonization and dispersed pigmented spores were detected (Fig. 6). These observations likely represent early or transitional stages of fungal development, where reproductive structures were limited but pigmented ellipsoidal spores were visible within the substrate matrix. Similarly, late-stage sporulation patterns were observed in horse and goat dung samples, characterized by low-density reproductive structures and scattered pigmented spores across the microscopic field (Fig. 7). These patterns likely represent post-maturation dispersal phases of coprophilous fungi in which fruiting bodies have already released spores into the surrounding environment. A combined microscopic overview of representative structures observed during this study is shown in Fig. 8. These include early *Pilobolus* development with rhizoidal trophocysts and phototropic sporangiophores (Figs. 8.1–3), pigmented *Ascobolus* ascospores and asci (Fig. 8.4), and apothecioid fruiting bodies consistent with *Ascobolus* developing on dung substrates (Fig. 8.5). Together, these observations confirm the presence of morphologically diverse coprophilous fungi associated with herbivore dung in KwaZulu-Natal and highlight the structural adaptations that enable these fungi to colonize, reproduce, and disperse within dung-based microhabitats.

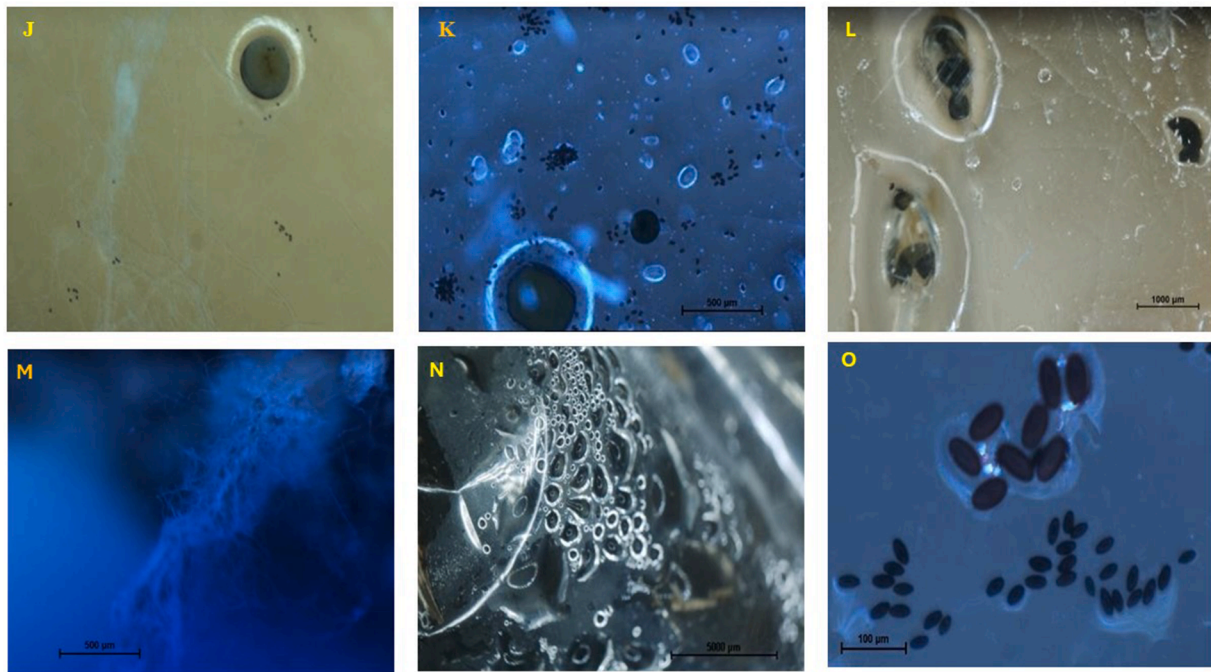


Fig. 5. A: (J–L) Zebra and white rhino dung showing mature embedded fruiting bodies and ascus- or sporangium-like structures. (M–O) White rhino and waterbuck dung showing extensive mycelial growth, clustered reproductive bodies and mature pigmented spores. *Ascobolus amoenus* Oudem. (J–K) Ascomata apothecioid, gregarious or scattered, semi-immersed in dung substrate, 500–700 µm diameter. Receptacle globose, becoming cupulate with age. Disc yellowish green to olive-green with numerous asci projecting from the hymenial surface. Hypothecium is thin and composed of globose hyaline cells. Medullary excipulum textura globulosa. Ectal excipulum consisting of pigmented angular cells. Paraphyses are cylindrical, hyaline, septate, and embedded within a mucilaginous matrix. Asci narrowly clavate, 8-spored, 180–250 × 45–60 µm. Ascospores ellipsoidal, smooth-walled, 30–40 × 20–25 µm, violet-brown at maturity. Observed in zebra and giraffe dung collected from Tala Game Reserve. This species differs from other *Ascobolus* taxa by its smaller ascospore size and distinctive greenish hymenial pigmentation. *Ascobolus immersus* Pers. **Fig. 5B:** (L–M) Ascomata apothecioid, solitary or gregarious, superficial to semi-immersed within the dung substrate, sessile, subglobose to cupulate, measuring approximately 600–1000 µm in diameter. Receptacle initially closed, becoming irregularly open at maturity, yellowish-brown to olive-brown in colour. Disc flat to convex with numerous protruding asci visible under a stereomicroscope. Hypothecium is thin and composed of isodiametric hyaline cells. Medullary excipulum consisting of textura globulosa. Ectal excipulum is composed of angular pigmented cells forming a protective outer layer. Paraphyses filiform, hyaline, septate, embedded in mucilaginous matrix, slightly exceeding asci, 2–4 µm wide. Asci broadly clavate to sacciform, 8-spored, unitunicate, 450–650 × 90–110 µm, operculate at apex. Ascospores ellipsoidal to subcylindrical, biserial, 50–60 × 25–35 µm, thick-walled, smooth, violet to dark purple at maturity and surrounded by a thin gelatinous sheath. Observed on moist chamber cultures of white rhinoceros and waterbuck dung collected from Phinda Game Reserve. The large pigmented ascospores surrounded by a gelatinous sheath are diagnostic features of *A. immersus*. This species is widely reported in herbivore dung ecosystems and represents one of the most common coprophilous fungi associated with large herbivores.

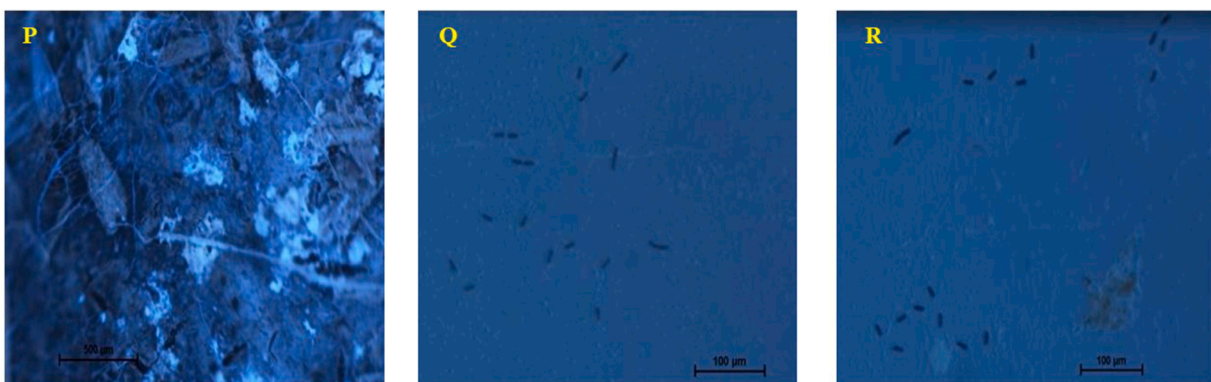


Fig. 6. (P–R) Impala, horse and goat dung show relatively sparse colonization with dispersed spores and limited fruiting development. Coprophilous fungal morphotype associated with impala dung. (P) Dung substrate with sparse superficial fungal colonization and plant debris. (Q) Dispersed pigmented ellipsoidal spores in a clear microscopic field. (R) Pigmented spores associated with a localized fragment of dung substrate. Scale bars: P = 500 µm; Q = 100 µm; R = 100 µm.

Overall, the morphological variation observed among *Ascobolus* taxa, including differences in apothecial pigmentation, ascospore size, ascus structure, and gelatinous sheath development, indicates the presence of multiple species within the sampled dung communities. These findings contribute valuable baseline information for understanding the diversity

of apothecioid coprophilous fungi in KwaZulu-Natal and provide a reference for future integrative taxonomic studies combining morphological and molecular approaches. Other *Ascobolus* collections showed pigmented spores and immature or partially resolved reproductive structures but lacked sufficient diagnostic characters for species-level

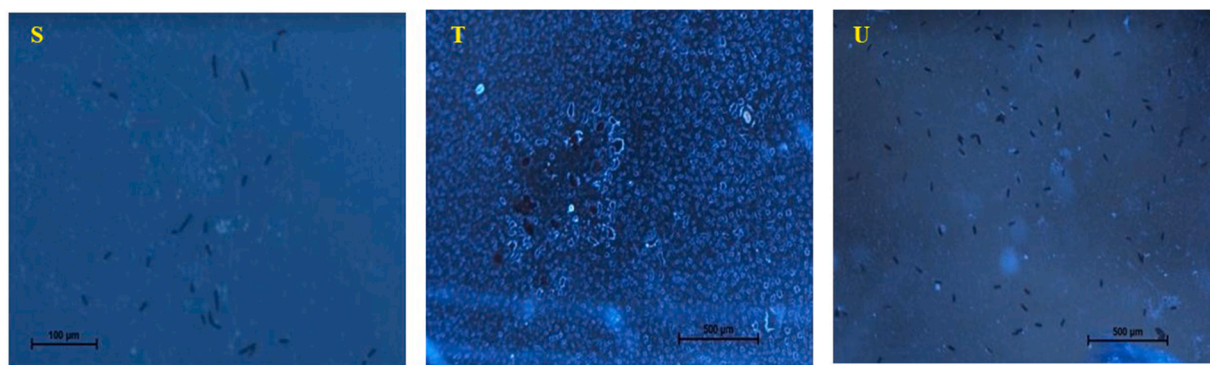


Fig. 7. (S–U) Horse and goat dung illustrating late-stage spore dispersal and low-density reproductive structures. Scale bars are indicated in individual panels. Coprophilous morphotypes showing late-stage sporulation and low-density reproductive development. (S) Sparse field of scattered pigmented spores. (T) Localized aggregation of rounded reproductive material within a dense, stained background. (U) Broad field of dispersed pigmented spores representing a late dispersal stage. Scale bars: S = 100 µm; T = 500 µm; U = 500 µm.

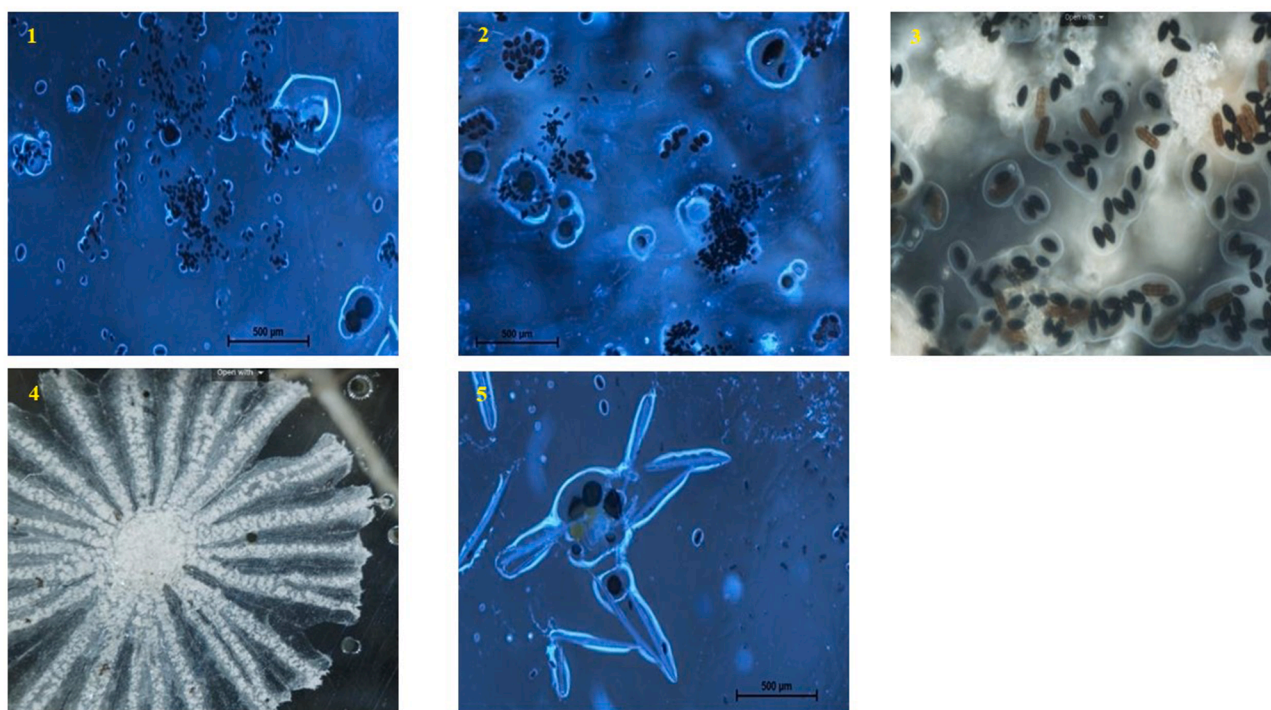


Fig. 8. (1) *Pilobolus* sp. with rhizoidal extensions and emerging phototropic sporangiophore. Trophocysts subglobose to ovoid, hyaline to slightly pigmented, semi-immersed in the dung substrate and connected to elongated rhizoidal extensions. (2) Sporangiophore bearing subsporangial vesicle and sporangium; Sporangiophores erect, long, cylindrical and unbranched, phototropic, arising from the trophocyst and extending upwards toward the light source. (3) Subsporangial vesicle inflated and smooth-walled, appearing hyaline to slightly orange-pigmented in transmitted light. Sporangia are hemispherical to subglobose, darkly pigmented at maturity, borne at the apex of the sporangiophore and supported by the inflated subsporangial vesicle. Columella is dome-shaped and smooth-walled. Sporangiospores numerous, smooth-walled, ellipsoidal to ovoid, pale yellow to brownish, approximately $7\text{--}10 \times 4\text{--}6 \mu\text{m}$. (4) Pigmented *Ascobolus* ascospores (and associated asci where visible); (5) apothecioid fruiting body consistent with *Ascobolus* developing on dung substrate. Scale bars: 500 µm.

assignment. These are reported as *Ascobolus* morphotype A–C and are treated as priority material for future molecular confirmation.

3.5. Diagnostic morphological characters

The diagnostic characters used to assign specimens to *Pilobolus* and *Ascobolus* are summarised in Table 3. The generic assignment was based on reproductive morphology rather than ecological occurrence alone. *Pilobolus* was distinguished by the presence of trophocysts, phototropic sporangiophores, inflated subsporangial vesicles, and terminal pigmented sporangia. *Ascobolus* was distinguished by apothecioid ascomata, operculate asci, pigmented thick-walled ascospores, and, in some

specimens, gelatinous spore sheaths (Table 3). Morphometric measurements were obtained from microscopic observations of fruiting bodies and spores using calibrated imaging software. For each structure, multiple measurements were recorded, and the resulting ranges are reported to capture observed variability. The selected diagnostic traits correspond to morphological characters traditionally used in coprophilous fungal taxonomy, including sporangiophore architecture, trophocyst morphology, ascus configuration, and ascospore size and shape (Aluoch et al., 2015; Bell, 1983; van Brummelen, 1967). Clear morphological differences were observed between the two genera. *Pilobolus* morpho-species were characterized by the presence of a swollen trophocyst embedded within the dung substrate, from which erect phototropic

Table 3
Diagnostic morphological characters used for morphology-based assignment of focal coprophilous fungi observed in herbivore dung from KwaZulu-Natal.

Taxon (assignment)	Diagnostic structure	Observed character state	Taxonomic significance	Identification confidence
<i>Pilobolus</i> sp. (morphospecies)	Trophocyst	Semi-immersed in dung, subglobose to ovoid, with rhizoidal extensions	Diagnostic for <i>Pilobolus</i> development on dung	Genus-level
<i>Pilobolus</i> sp. (morphospecies)	Sporangiophore	Erect, hyaline, unbranched, phototropic	Characteristics of <i>Pilobolus</i>	Genus-level
<i>Pilobolus</i> sp. (morphospecies)	Subsporangial vesicle	Inflated, hyaline to faintly pigmented, supporting sporangium	Inflated, ovoid to elliptical vesicle supporting the sporangium. Important generic character	Genus-level
<i>Pilobolus</i> sp. (morphospecies)	Sporangium	Dark, hemispherical to subglobose, terminal	Smooth-walled, typically ellipsoid; dimensions consistent with reported <i>Pilobolus</i> ranges. Consistent with ballistic spore discharge	Genus-level
<i>Ascobolus</i> sp. (morphospecies)	Ascomata	Apothecioid, cupulate to discoid, superficial to semi-immersed	Cupulate to discoid apothecia; superficial to semi-immersed in dung. Diagnostic for <i>Ascobolus</i> and related Ascobolaceae	Genus-level
<i>Ascobolus</i> sp. (morphospecies)	Asci	Operculate, clavate to sacciform, usually 8-spored where mature	Clavate, 8-spored, operculate; typical of <i>Ascobolus</i> . Important character for <i>Ascobolus</i> identification	Genus-level / cf.
<i>Ascobolus</i> sp. (morphospecies)	Ascospores	Pigmented, thick-walled, ellipsoid to subcylindrical	Pigmented, thick-walled; frequently surrounded by a gelatinous sheath. Important for species comparison	Genus-level / cf.
<i>Ascobolus</i> morphospecies	Gelatinous sheath	Present in some mature ascospores	Useful in comparison with taxa such as <i>A. immersus</i>	Species-comparison character

sporangiophores arise. These sporangiophores terminate in a subsporangial vesicle supporting a dark, hemispherical sporangium that contains smooth-walled sporangiospores. These structural features are consistent with the characteristic morphology associated with the genus and reflect its specialized ballistic spore-dispersal mechanism (Aluoch et al., 2017; Bell, 1983).

In contrast, *Ascobolus* morphotypes formed apothecioid ascomata that were cupulate to discoid and typically superficial to semi-immersed in the dung substrate. The hymenial layer contained clavate, operculate asci bearing eight pigmented ascospores. The ascospores were thick-walled and often surrounded by a gelatinous sheath, a diagnostic feature commonly used to distinguish *Ascobolus* species (Bell, 1983, van Brummelen, 1967). Variability in ascospore size and ascus dimensions

among samples suggests the presence of multiple *Ascobolus* taxa within the studied dung communities. These morphometric observations support the morphological assignment of the recovered taxa and provide a quantitative framework for distinguishing coprophilous fungi present in herbivore dung from KwaZulu-Natal. The measurements presented here provide a baseline reference for future taxonomic and ecological studies of dung-associated fungi in South African ecosystems. The measurements reported here provide a morphology-based reference dataset for the focal taxa recovered in this study. However, because no ITS barcode or multilocus phylogenetic data were generated, the taxonomic assignments should be interpreted as morphology-based identifications. Species names are therefore used conservatively, with cf. or morphotype designations where appropriate. To facilitate comparison of the

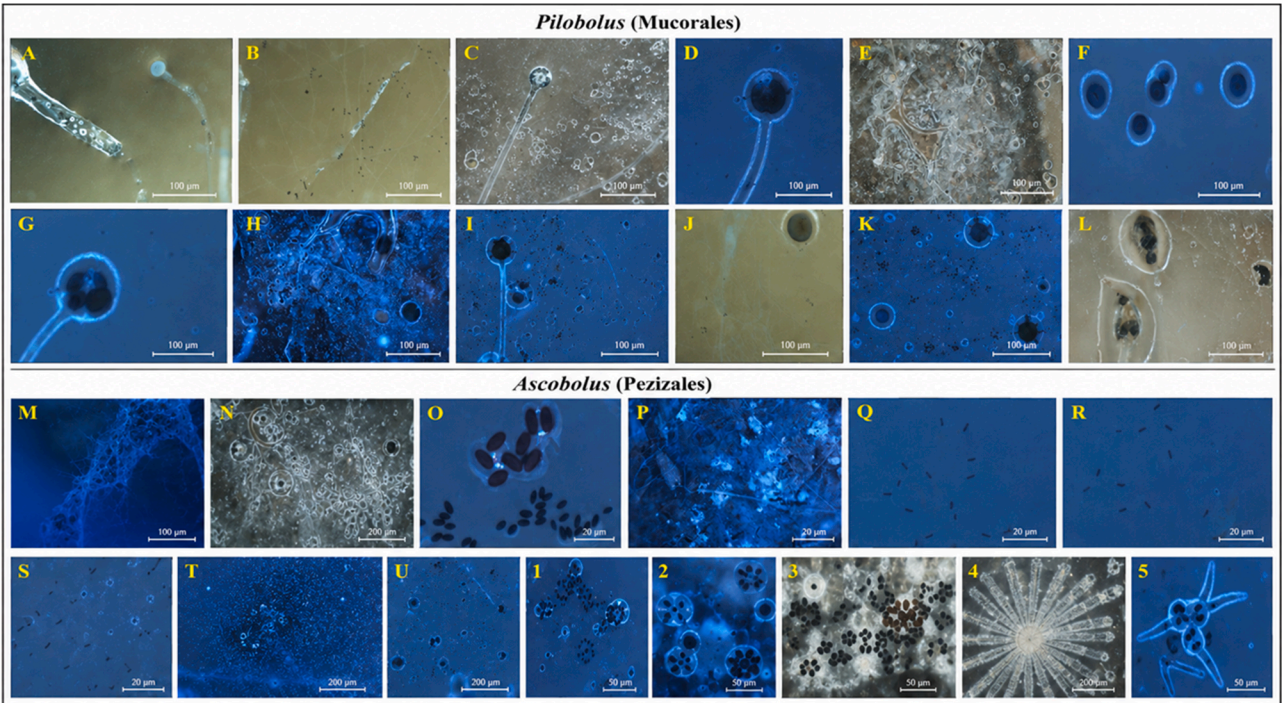


Fig. 9. Representative diagnostic microscopic structures used for morphology-based identification of coprophilous fungi recovered from herbivore dung in KwaZulu-Natal, South Africa. (A) *Pilobolus* trophocyst embedded within the dung substrate with rhizoidal attachment. (B) Unbranched phototropic sporangiophore arising from the trophocyst. (C) Inflated subsporangial vesicle supporting the terminal sporangium. (D) Mature hemispherical sporangium containing ellipsoidal sporangiospores. (E) *Ascobolus* apothecioid fruiting body developing on the dung surface. (F) Operculate ascus containing eight mature ascospores. (G) Pigmented, thick-walled *Ascobolus* ascospores used as diagnostic characters for morphology-based identification. Scale bars are shown in each panel.

principal diagnostic features, representative microscopic structures observed during this study are presented together in (Figure 9), illustrating the key morphological characters used to distinguish *Pilobolus* and *Ascobolus* based on standard taxonomic criteria (Bell, 1983; van Brummelen, 1967; Doveri, 2016).

The morphometric analysis revealed clear diagnostic differences between members of the genera *Pilobolus* and *Ascobolus*. The *Pilobolus* species were distinguished by the presence of a swollen trophocyst embedded within the dung substrate and long phototropic sporangio-phores terminating in hemispherical sporangia, features characteristic of coprophilous zygomycetes adapted for ballistic spore dispersal (Krug et al., 2004; Bell, 1983). In contrast, *Ascobolus* species form apothecioid ascomata containing clavate, operculate asci and pigmented, thick-walled ascospores, often surrounded by a gelatinous sheath. These traits are diagnostic of members of the Ascobolaceae and are widely used for taxonomic identification within the genus (Richardson, 2001; van Brummelen, 1967).

Among the taxa identified, *Ascobolus immersus* exhibited the largest ascospore dimensions, whereas *A. amoenus* produced comparatively smaller ellipsoidal spores. Within the zygomycetous taxa, *Pilobolus crystallinus* (Fig. 2A, B, C) displayed the longest sporangiophores and largest trophocysts. Overall, the morphometric ranges recorded fall within previously reported diagnostic limits for coprophilous fungi associated with herbivore dung, supporting the reliability of the morphological assignments made in this study (Aluoch et al., 2017; Krug et al., 2004; Bell, 1983).

3.6. Comparative morphology of selected coprophilous fungi

The morphological characteristics of the coprophilous fungi isolated from herbivore dung samples in KwaZulu-Natal are summarized in (Table 4). The taxa identified in this study included members of the genera *Pilobolus* and *Ascobolus*, which represent typical early and intermediate colonizers during coprophilous fungal succession (Krug et al., 2004; Richardson, 2001; Weber and Webster, 1998). Morphological identification was based on diagnostic structures such as trophocysts, sporangiophores, sporangia, asci, ascomata, and ascospore

morphology following established taxonomic keys and comparative descriptions of dung-associated fungi (Calaga et al., 2024; Ojwach et al., 2022; Doveri, 2016; Aluoch et al., 2015; Bell, 1983; van Brummelen, 1967).

Distinct morphological differences were observed between the two genera. Species of *Pilobolus* were characterized by erect sporangiophores arising from swollen trophocysts embedded in the dung substrate and terminating in hemispherical sporangia, a structural arrangement associated with the genus's well-known ballistic spore dispersal mechanism (Aluoch et al., 2017; Bell and Mahoney, 2007; Krug et al., 2004; Bell, 1983). Among the *Pilobolus* collections, morphotypes comparable to *P. crystallinus* showed large trophocysts, erect phototropic sporangiophores, inflated subsporangial vesicles, and dark terminal sporangia. Other *Pilobolus* collections showed smaller reproductive structures and are retained as *Pilobolus* morphospecies pending molecular confirmation.

In contrast, *Ascobolus* species produced apothecioid ascomata containing clavate, operculate asci and pigmented ascospores often surrounded by a gelatinous sheath, features that are diagnostic of the Ascobolaceae and widely used in species delimitation (Doveri, 2016; Bell and Mahoney, 2007). The morphometric and structural traits recorded in this study are consistent with previously reported descriptions of coprophilous fungi associated with herbivore dung across Africa and other regions (Ribeiro Melo et al., 2026; Ndozi et al., 2025; Subrahmanyam and Sambamurthy, 2023; Basumatary et al., 2020; Kaur et al., 2019; Portillo et al., 2012; Mungai et al., 2012; Mungai et al., 2011). Variation in sporangiophore length, ascospore size, and fruiting body morphology further supports the presence of multiple coprophilous taxa adapted to different dung substrates and host-associated microhabitats. Finally, among the *Ascobolus* collections, morphotypes comparable to *A. amoenus* were distinguished by smaller ellipsoid ascospores and greenish hymenial pigmentation, whereas morphotypes comparable to *A. immersus* showed larger ascospores and a gelatinous sheath. Additional *Ascobolus* morphotypes were retained at the genus level because mature asci, paraphyses, or fully resolved ascospores were not consistently observed.

Table 4

Conservative comparative morphological assignments of selected *Pilobolus* and *Ascobolus* morphospecies recovered from herbivore dung in KwaZulu-Natal.

Conservative assignment	Previous assignment in the original manuscript	Diagnostic structures observed	Spore size/morphology	Dung source	Revision rationale
<i>Pilobolus cf. crystallinus</i>	<i>Pilobolus crystallinus</i>	Large trophocyst, rhizoids, long phototropic sporangiophore, inflated subsporangial vesicle, dark terminal sporangium	Smooth ellipsoid to ovoid sporangiospores, approx. 7-10 × 4-6 µm	Bushbuck, zebra	Treated as cf. because morphology is consistent, but molecular confirmation is absent
<i>Pilobolus</i> morphospecies A	<i>Pilobolus pullus</i>	Smaller trophocyst, shorter sporangiophore, ovoid vesicle, terminal sporangium	Smooth ellipsoid sporangiospores, approx. 8-10 × 4-6 µm	Bovine, giraffe, zebra	Species-level assignment not retained without stronger diagnostic evidence
<i>Pilobolus</i> morphotype B	<i>Pilobolus heterosporus</i>	Shorter sporangiophore, ellipsoid trophocyst, terminal sporangium	Variable ellipsoid spores, approx. 5.6-10.1 × 5.1-6.1 µm	White rhinoceros, waterbuck	Retained as the morphotype because the original assignment requires stronger comparative and molecular support
<i>Ascobolus cf. amoenus</i>	<i>Ascobolus amoenus</i>	Apothecioid ascomata, greenish-yellow to olive hymenium, clavate asci, pigmented ellipsoid ascospores	Approx. 30-40 × 20-25 µm	Zebra, giraffe	Treated as cf. because morphology is consistent, but sequence data are absent
<i>Ascobolus</i> morphotype A	<i>Ascobolus bistitii</i>	Apothecioid ascomata, broad asci, pigmented ellipsoid ascospores	Approx. 53-58 × 30-33 µm	White rhinoceros, zebra, waterbuck	Species name removed pending stronger diagnostic comparison
<i>Ascobolus</i> morphotype B	<i>Ascobolus calesco</i>	Semi-immersed yellow-brown apothecioid ascomata, large asci, pigmented ascospores	Approx. 48-57 × 27-33 µm	Bushbuck	Species name removed because diagnostic distinction from related taxa is not fully demonstrated
<i>Ascobolus cf. immersus</i>	<i>Ascobolus immersus</i>	Large apothecioid ascomata, broadly clavate to sacciform asci, large pigmented ascospores with gelatinous sheath	Approx. 50-60 × 25-35 µm	White rhinoceros, waterbuck, zebra	Treated as cf. because morphology is consistent, but molecular confirmation is absent
<i>Ascobolus</i> morphotype C	<i>Ascobolus</i> sp. 4	Immersed to semi-immersed apothecioid structures; asci incompletely resolved	Approx. 35-45 × 20-25 µm where measurable	Bushbuck-like browsing dung	Retained as morphotype because mature diagnostic structures were incomplete

4. Discussion

This study provides a morphology-based baseline assessment of selected coprophilous fungi associated with herbivore dung in KwaZulu-Natal, South Africa. The revised interpretation is intentionally conservative. The dataset documents observed occurrence and morphology of focal *Ascobolus* and *Pilobolus* taxa under moist-chamber incubation, but it does not claim to represent complete coprophilous fungal diversity, nor does it test statistically supported host- or site-level ecological hypotheses. The observed fruiting sequence broadly followed the classical succession described for dung fungal communities, with *Pilobolus*-like Mucorales appearing early and *Ascobolus*-like apothecial Ascomycota appearing later. This pattern supports the ecological interpretation that rapidly developing zygomycetous fungi exploit fresh dung during early decomposition, whereas apothecial Ascomycota develop later as the substrate changes. However, because incubation conditions and sample numbers were uneven across host dung types, these observations should be interpreted as descriptive baseline patterns (Krug et al., 2004; Richardson, 2001; Weber and Webster, 1998). In the present study, *Pilobolus* morphospecies repeatedly appeared during the first week of incubation, whereas *Ascobolus* morphotypes emerged later. This pattern is consistent with nutrient-driven succession in dung ecosystems, in which early “sugar fungi” are gradually replaced by taxa capable of degrading more recalcitrant structural plant polymers such as cellulose and hemicellulose (Sarrocio, 2016; Dix, 2012; Richardson, 2001; Harper and Webster, 1964).

4.1. Host diet, digestive physiology, and dung “microhabitat filtering”

Dung is a heterogeneous and dynamic microhabitat whose physical and chemical properties are strongly influenced by host diet and digestive physiology. Herbivore feeding strategies determine the composition of plant residues, microbial communities, and physico-chemical conditions present in dung deposits (Holter, 2016; Portillo et al., 2012; Shahack-Gross, 2011). The observed variation in morphospecies richness among herbivore dung types (Fig. 1B) suggests that substrate characteristics associated with different host species influence the establishment and development of coprophilous fungi. Hindgut

fermenters such as rhinoceroses and equids typically excrete dung containing relatively high amounts of partially digested plant material, which can extend substrate availability and promote prolonged fungal colonization. In contrast, ruminant digestion often results in more finely processed plant matter and altered nutrient profiles that may influence fungal community assembly (Min and Solaiman, 2018). Grazers such as buffalo, zebra and white rhinoceros consume predominantly grasses that produce fibrous dung rich in cellulose and hemicellulose. These substrates generally retain moisture longer and persist in the environment, providing favourable conditions for successive colonisation by coprophilous fungi. In contrast, browsers such as giraffe and bushbuck consume woody vegetation containing higher concentrations of lignin and secondary metabolites, which may influence dung chemistry and consequently fungal community composition. These dietary differences likely contributed to the variation in morphospecies richness observed among herbivore hosts in the present study.

In addition to diet-derived differences, dung pH, moisture content, and fibre composition collectively influence which fungal communities successfully establish fruit (Kuthubutheen and Webster, 1986; Abdullah, 1982). In the present study, rhinoceros dung represented the most intensively sampled substrate and consistently supported fungal fruiting during incubation. This observation likely reflects its relatively fibrous structure and high moisture retention, which together provide a stable microenvironment conducive to fungal development. Similar patterns have been reported in other studies of coprophilous fungi. For example, Doveri (2016) documented high species richness of coprophilous fungi on dung from large wild herbivores, including deer and horses, while Krug et al. (2004) reported that the prolonged persistence and nutrient-rich nature of large herbivore dung supported a diverse assemblage of ascomycetes and zygomycetes. Likewise, van Asperen (2017) found that larger dung parts remained suitable for fungal colonisation over extended periods, allowing successive waves of coprophilous fungi to develop. More recently, Calaça et al. (2024) demonstrated that dung from large grazing mammals supported a higher richness and succession of coprophilous fungi than smaller, rapidly desiccating dung substrates.

The patterns observed in the present study are broadly consistent with reports of coprophilous fungal communities from other regions of

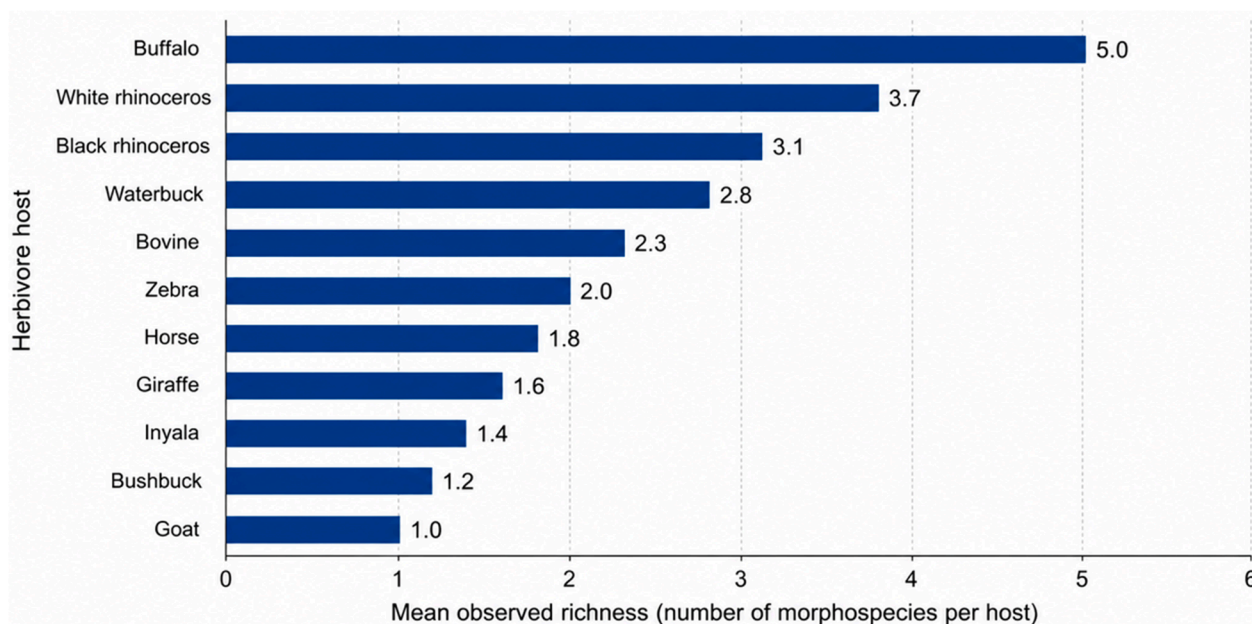


Fig. 1B. Mean observed richness of morphology-based *Ascobolus* and *Pilobolus* morphospecies recovered from dung of domestic and wild herbivores collected across KwaZulu-Natal, South Africa. Richness was calculated as the number of morphologically distinguishable focal morphospecies recovered from each herbivore host following moist-chamber incubation. Buffalo dung supported the highest observed richness, whereas goat dung supported the lowest. Values are presented as descriptive summaries because sampling effort differed among herbivore hosts and no inferential statistical analyses were performed.

the world, although differences in species richness, dominant genera and host associations reflect variations in climate, herbivore assemblages and sampling approaches. In Kenyan studies, [Aluoch et al. \(2015, 2017\)](#) demonstrated that *Pilobolus* and *Ascobolus* are among the most frequently encountered coprophilous genera on herbivore dung, particularly from large grazing mammals, consistent with the dominance of these genera observed in the present study. Similar observations have been reported in East Africa, where surveys in Kenya documented diverse assemblages of *Ascobolus*, *Pilobolus*, *Podospira* and *Sordaria* associated with wildlife and livestock dung, highlighting the influence of host identity and substrate characteristics on fungal colonisation ([Aluoch et al., 2017](#)). In Central Africa, studies from Cameroon likewise reported that herbivore dung supports a succession of morphologically distinct coprophilous fungi, with species composition influenced by dung moisture, vegetation type and seasonal conditions ([Nchanji and Plumptre, 2001](#)). Comparable patterns have also been described in Brazil, where *Ascobolus*, *Pilobolus*, *Podospira* and *Coprinopsis* dominate dung fungal communities in tropical grazing ecosystems ([Ribeiro Melo et al., 2026](#)), and in Asia, where fibrous dung from cattle and buffalo consistently supported higher fungal richness and prolonged successional development than dung from smaller herbivores ([Kuthubutheen and Webster, 1986](#); [Abdullah, 1982](#)). Across Europe, long-term investigations have similarly demonstrated that large herbivore dung supports characteristic successional sequences in which rapidly growing Mucorales, particularly *Pilobolus*, colonise fresh dung before being replaced by ascomycetous taxa including *Ascobolus*, *Podospira* and *Sordaria* during later stages of decomposition ([van Asperen, 2017](#)). Collectively, these studies indicate that although the taxonomic composition of coprophilous fungal communities varies geographically, substrate quality, host diet, dung persistence and successional stage remain the principal ecological drivers shaping fungal richness and community composition across terrestrial ecosystems.

4.2. Ecological roles and dispersal adaptations

Coprophilous fungi play a central role in nutrient cycling within terrestrial ecosystems by accelerating the decomposition and mineralization of herbivore dung ([Masunga et al., 2006](#)). Through enzymatic degradation of plant-derived organic matter, these fungi contribute to the release of nutrients back into soils and support the functioning of dung-based food webs ([Basumatary et al., 2020](#); [Sarrocco, 2016](#)). The genera documented in this study represents important functional components of this ecological process. The repeated occurrence of *Pilobolus* is ecologically significant because this genus possesses one of the most specialized dispersal mechanisms among coprophilous fungi. Its phototropic sporangiophores orient toward light and explosively discharge sporangia over considerable distances from the dung substrate. This ballistic dispersal mechanism enables spores to reach surrounding vegetation, increasing the likelihood that they will be ingested by grazing herbivores and subsequently pass through the digestive tract to colonize new dung deposits ([Krug et al., 2004](#); [Bell, 1983](#)). In addition to its ecological role in fungal dispersal, *Pilobolus* has veterinary relevance through its documented association with the dispersal of lung-worm larvae (*Dictyocaulus* spp.), which may contribute to parasitic bronchitis in grazing animals ([Mitchell et al., 2025](#)). Establishing baseline records of *Pilobolus* occurrence in the South African dung ecosystem, therefore, contributes not only to fungal biodiversity documentation but also to understanding potential interactions between fungal dispersal mechanisms and parasite ecology in mixed wildlife-livestock landscapes. Species of *Ascobolus* exhibit a different set of adaptations linked to their reproductive strategy and dispersal ecology. The apothecoid ascomata and pigmented, thick-walled ascospores characteristic of the genus are well-suited for survival under exposed environmental conditions. Pigmentation may protect spores from ultraviolet radiation on exposed vegetation, while gelatinous sheaths surrounding the spores may facilitate adhesion to plant surfaces or soil particles, enhancing the

likelihood of ingestion by grazing animals ([Richardson, 2001](#); [Bell, 1983](#); [van Brummelen, 1967](#)). These traits reflect the evolutionary pressures imposed by the ephemeral nature of dung substrates and the need for efficient dispersal between successive dung deposits. In mixed wildlife-livestock systems such as KwaZulu-Natal, *Pilobolus*-mediated dispersal of *Dictyocaulus* larvae may facilitate parasite transmission between domestic livestock and wild herbivores where grazing areas overlap. Although parasite transmission was not investigated in this study, documenting the occurrence of *Pilobolus* provides valuable baseline information for future studies integrating fungal ecology with veterinary parasitology and wildlife health.

4.2.1. Methodological implications: morphology vs cryptic diversity

Although morphology-based identification remains fundamental in coprophilous mycology, recent studies highlight the increasing importance of integrating molecular tools into fungal taxonomy ([Miranda et al., 2020](#); [Herrera et al., 2011](#); [Edwards et al., 2017](#)). Morphological traits such as ascospore size, ascus configuration, and sporangiophore architecture provide critical diagnostic information; however, species boundaries within several coprophilous genera, including *Pilobolus*, may be obscured by morphological convergence and cryptic diversity ([Calaça et al., 2024](#); [Subrahmanyam and Sambamurty, 2023](#)). Consequently, the morphospecies documented in this study should be considered a robust morphological baseline for KwaZulu-Natal dung ecosystems, while also representing priority targets for future integrative taxonomic work. Incorporating molecular markers such as the ITS region and additional phylogenetic loci will enable confirmation of species identities, clarification of phylogenetic relationships, and detection of cryptic lineages that cannot be resolved using morphology alone ([Doveri, 2016](#); [Bridge et al., 2008](#)). Such integrative approaches are increasingly recognized as essential for accurately documenting fungal biodiversity and understanding ecological patterns in dung-associated fungal communities. Future studies should also integrate ITS DNA barcoding with the large subunit ribosomal RNA gene for phylogenetic backbone (LSU), RNA polymerase II second largest subunit for resolving relationships (RPB2) and Translation elongation factor 1- α for species-level resolution (TEF1- α) sequence data where appropriate to improve species delimitation and detect cryptic diversity within morphologically similar coprophilous fungi.

4.3. Contribution and future perspectives

This study provides one of the first structured morphological baselines for coprophilous fungi associated with herbivore dung across multiple host species in KwaZulu-Natal. By documenting the occurrence and morphological characteristics of *Ascobolus* and *Pilobolus* taxa across diverse dung substrates, the findings contribute valuable baseline data for regional fungal biodiversity assessments. Such baseline information is essential for long-term monitoring of fungal communities in wildlife reserves and agro-ecological landscapes, where interactions between wildlife, livestock, and environmental change may influence dung-associated microbial ecosystems ([Ribeiro Melo et al., 2026](#); [Ndozi et al., 2025](#); [Ojwach, 2018](#); [Ojwach et al., 2022](#)). Future research should extend these observations by integrating molecular phylogenetic approaches with ecological surveys to better resolve species diversity and functional roles within dung ecosystems. In addition, studies examining seasonal variation, host-specific fungal assemblages, and interactions between fungi, dung beetles, and other dung-associated organisms would provide further insights into the complexity of dung food webs and their contribution to ecosystem functioning. Overall, the results presented here demonstrate that herbivore dung in KwaZulu-Natal supports a diverse assemblage of coprophilous fungi with distinct morphological adaptations for colonization, reproduction, and dispersal. Establishing such regional baselines is an essential step toward a more comprehensive understanding of fungal biodiversity and the ecological processes governing dung decomposition in southern

African ecosystems.

4.4. Limitations and taxonomic caution

A major limitation of this study is the absence of ITS barcode sequences and phylogenetic analyses. Molecular confirmation is increasingly important in fungal taxonomy, especially for morphologically similar or cryptic taxa. Therefore, the species-level assignments presented here are conservative. Collections with strong morphological similarity to published species are reported as cf. taxa, whereas collections lacking mature diagnostic structures are retained as morphotypes.

A second limitation is the uneven sampling design across dung hosts and sites. Consequently, richness patterns are presented descriptively and should not be interpreted as statistically tested ecological differences. Future studies should include balanced replication across host species, seasons, and sites, together with environmental measurements such as dung moisture, pH, fibre content, and nutrient composition. Despite these limitations, the study contributes useful baseline information by linking focal coprophilous morphotypes to dung source, site, diagnostic morphology, and voucher status. This provides a foundation for future integrative taxonomic work combining morphology, voucher specimens, DNA barcoding, and phylogenetic analysis.

5. Conclusion

Coprophilous fungi isolated from herbivore dung in the KwaZulu-Natal Province exhibited characteristic successional development under moist-chamber incubation, with early colonization by *Pilobolus* morphospecies followed by the emergence of *Ascobolus* morphotypes at later stages. This study documents morphology-based records of focal coprophilous fungi assigned to *Ascobolus* and *Pilobolus* from domestic and wild herbivore dung in KwaZulu-Natal, South Africa. Under moist-chamber incubation, *Pilobolus* morphospecies generally appeared during early incubation, whereas *Ascobolus* morphotypes appeared later, consistent with known patterns of dung fungal succession. Diagnostic structures observed included trophocysts, phototropic sporangiophores, subsporangial vesicles, and sporangia in *Pilobolus*, and apothecioid ascomata, operculate asci, and pigmented ascospores in *Ascobolus*. Because molecular data were not generated, taxonomic assignments are treated conservatively as cf. taxa or morphotypes where appropriate. The study should therefore be viewed as a baseline morphological survey rather than a definitive taxonomic revision. Future work should combine voucher-based morphology with ITS barcoding, multilocus phylogenetics, balanced ecological sampling, and environmental metadata to resolve species boundaries and test host- or site-level ecological patterns. Furthermore, we recommend future research that integrates morphological observations with molecular approaches to resolve cryptic diversity and refine taxonomic resolution. Such integrative studies will strengthen ecological interpretation and advance our understanding of the roles coprophilous fungi play in nutrient cycling and dung ecosystem functioning in southern Africa.

Data availability

Raw morphometric measurements, isolate metadata, taxonomic assignments, and voucher information are provided in **Supplementary Table S1**. Representative voucher material has been deposited at the University of KwaZulu-Natal under accession number (MH445969). No DNA sequence data were generated for this study. Therefore, no GenBank accession numbers are associated with the present manuscript.

CRediT authorship contribution statement

Jeff D. Ojwach: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Samson Mukaratirwa:** Writing – review & editing, Supervision, Resources,

Project administration, Funding acquisition. **Taurai Mutanda:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.sajb.2026.08.029](https://doi.org/10.1016/j.sajb.2026.08.029).

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