

Coprophilous fungi from dung of the Greater One-Horned Rhino in Kaziranga National Park, India and its implication to paleoherbivory and paleoecology

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Abstract

Fungal spores, especially those of coprophilous fungi, are present in dung middens of *Rhinoceros unicornis* (greater one-horned rhinoceros) in both forest and grassland areas of the Kaziranga National Park, India. The presence of coprophilous fungi on rhino dung, chiefly *Sporormiella*, *Saccobolus*, *Ascodesmis*, *Cercophora*, and *Sordaria*, is documented for the first time. The *Sporormiella*–*Ascodesmis*–*Saccobolus* assemblage is abundant and characterizes the rhino dung in forest and grassland areas. The presence of coprophilous fungi spores allows for an examination of the relationship between rhinoceros ecology and the flora and other fauna in the region. The overall dataset is useful in interpreting the present and past distribution of rhino and other associated animals based on the relative abundance of different types of coprophilous fungi spores and their relationship to paleoherbivory and paleoecology in India and adjoining areas.

Keywords: Ecology; Endangered species; Fungal spectra; Grassland; Megaherbivores; Dung midden; *Rhinoceros unicornis*

INTRODUCTION

During the late Pleistocene most of the megafauna, both herbivores and carnivores, became extinct on all the major continents (Martin, 1967, 1984; Barnosky et al., 2004). Previous studies on the cause of this megafaunal extinction in different parts of the globe have tended to focus on two primary causes, climatic and anthropogenic (Martin, 1973; MacPhee, 1999; Miller et al., 1999; Grayson and Meltzer, 2002). The current rate of population reduction and potential extinction of herbivores and carnivores in the wild is a major global ecological issue. Currently about 60% of the large herbivorous animals are now threatened with possible extinction (Ripple et al., 2015). Southeast Asia contains the world's highest number of threatened mammals (Schipper et al., 2008), with regional faunas experiencing ongoing range reductions and extinctions driven by human activities (Brook et al., 2014). In India, a preliminary report on the status of the mega-herbivores, including the greater one-horned rhinoceros (*Rhinoceros unicornis*, Linnaeus, 1758;

also known as the Indian rhino) describes the high probability of their local extinction (Karanth et al., 2016).

The study of the dung of individual species is an important source of information on food preferences, habitat utilized, and ecology in general. Studies of fungal remains preserved in peat and lake sediments can complement palynodata in interpreting the paleovegetation and past climate in the region (van Geel, 1978, 1986, 2001; van Geel et al., 1981, 1989; Gill et al., 2009; Cugny et al., 2010; Feeser and O'Connell, 2010; Kramer et al., 2010; Montoya et al., 2010; Mudie et al., 2010) and in archaeological sites (van Geel et al., 2003; Zong et al., 2007; Gauthier et al., 2010; McAndrews and Turton, 2010; Rattighieri et al., 2013; Revelles et al., 2016). Studies have been carried out on coprophilous fungi in surface and sedimentary soil profiles to document or infer the former presence, and subsequent decline of, herbivorous animals in a region (Davis, 1987; Burney et al., 2003; Barnosky et al., 2004; Robinson et al., 2005; Raper and Bush, 2009). Feranec et al. (2011) noted the need for more studies to better understand *Sporormiella* as a proxy and to identify whether particular taxa are only present on the dung of specific herbivores. Here we document the presence, types, and abundance of the coprophilous fungi and associated spores present in the dung of *Rhinoceros unicornis* in Kaziranga National Park, India.

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The generated dataset can serve as a critical proxy to document the former existence of rhinoceroses in a region through samples collected from the surface and sediment/soil sediments that preserve coprophilous fungi.

Rhinoceros unicornis is one of the largest living mega-herbivores in the world and is now a critically endangered species (Poudyal et al., 2009). One of the unique behaviors of rhinoceroses, including *Rhinoceros unicornis* is to consistently use the same location for their daily excretion and multiple individuals may deposit dung at this site or midden over several years. The historical distribution of *Rhinoceros unicornis* includes habitats in northern and central India, and Pakistan (Rao, 1947; Banerjee and Chakraborty, 1973; Mathpal, 1978), but *Rhinoceros unicornis* is absent in these regions today. The current distribution of *Rhinoceros unicornis*, now restricted to a few areas in the Assam region of India and Nepal, is considerably smaller than the historical distribution of the species.

STUDY SITE, FLORA, AND FAUNA

Kaziranga National Park is an ideal place for the investigation of *Rhinoceros unicornis* in its natural habitat and to understand the ecology of the species. The park has the highest population of *Rhinoceros unicornis* in the world and the population has been increasing at a positive rate from 366 individuals in 1966 to 2048 individuals in 2009 (Medhi and Saha, 2014). In 2015 the rhino population was 2401 (Sharma, 2016). The park lies between 26°32' and 26°47' N, and 93°07' E to 93°38' E, at an elevation between 45–90 m above sea level (Fig. 1). The vegetation is mainly tropical, semi-evergreen, deciduous, savannah, and grassland (Champion and Seth, 1968). A list of flora in the park is provided in Table 1.

The Kaziranga National Park has rich and diverse vertebrate fauna. Among the herbivores, along with the *Rhinoceros unicornis* (one-horned rhino; Fig. 2a), the other

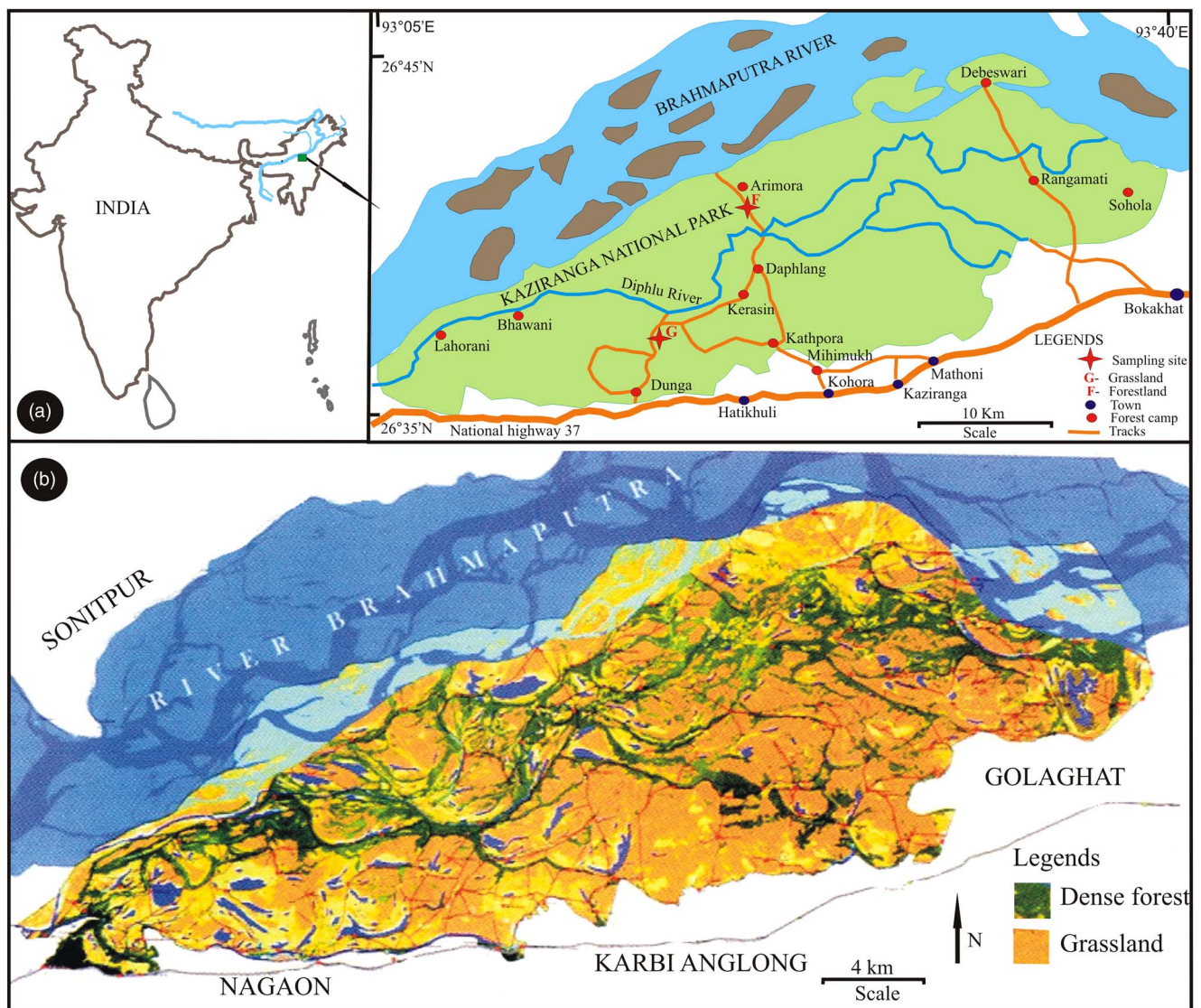


Figure 1. (color online) (a) Location of the study area. (b) Land cover map of the Kaziranga National Park, India (modified after Das et al., 2014)

Table 1. Plant taxa present in the Kaziranga National Park, India.

Scientific name
Angiosperms
<i>Albizia lebbbeck</i> (L.) Benth.
<i>Acacia catechu</i> (L. f.) Willd.
<i>Mesua ferrea</i> Linn.
<i>Cinnamomum bijolghota</i> (Buch.-Ham.)
<i>Magnolia hodgsonii</i> (Hook.f. & Thomson) H. Keng
<i>Aphanamixis polystachya</i> (Wall.) R.N.Parker
<i>Dillenia indica</i> Linn.
<i>Salmalia malabarica</i> (DC.) Schott & Endl.
<i>Terminalia billirica</i> (Gaerth.) Roxb.
<i>Syzygium cumini</i> (L.) Skeels.
<i>Duabanga grandiflora</i> (Roxb. Ex DC.) Walpers
<i>Lagerstroemia speciosa</i> (L.) Pers.
<i>Grewia serrulata</i> DC.
<i>Erianthus ravennae</i> Linn.
<i>Pennisetum purpureum</i> Schumach.
<i>Phragmites karka</i> (Retz.) Trin. ex Steud.
<i>Arundo donax</i> Linn.
<i>Imperata cylindrica</i> Linn.
<i>Saccharum procerum</i> Roxb.
<i>Polygonum orientale</i> Linn.
<i>Cyperus rotundus</i> Linn.
<i>Sagittaria segitifolia</i> Linn.
<i>Eichhornia crassipes</i> (Mart.) Solma
<i>Vallisneria spiralis</i> Linn.
<i>Nymphaea nouchali</i> Burm. f.
<i>Euryle ferox</i> Salisb.
<i>Myriophyllum indicum</i> Willd.
<i>Nymphoides indica</i> (L.) Kuntze
Ferns
<i>Lycopodium clavatum</i> Linn.
<i>Dryopteris filix-mas</i> (L.) Schott
<i>Gleichenia dichotoma</i> (Thunb.) Hook.
<i>Adiantum caudatum</i> Klotzsch
<i>Drynaria rigidula</i> (Sw.) Bedd.
<i>Lygodium japonicum</i> (Thunb.) Sw.
<i>Polypodium microrrhizoma</i> Clarke ex Bak.
<i>Diplazium esculatum</i> (Retz.) Sw.
<i>Blechnum occidentale</i> Linn.

common mammalian herbivores are *Bubalus arnee*, *Elephas maximus*, *Rusa unicolor*, and *Rucervus duvaucelii*.

CLIMATE AND SOIL

The climate of the region is controlled by the southwest and northeast monsoons; it is hot and humid during the summer, and cold and dry during winter. The maximum temperature ranges from a minimum of 4°C during winter up to 37°C in summer. The relative humidity is very high and ranges from 75 to 86%. The annual rainfall ranges from 1800 to 2600 mm, and annual flooding is very common in the Kaziranga National Park. The soil composition varies from site to site and includes sandy loam soil in forests, sandy soil in

grassland, and clayey soil in the swamp and water bodies (Das et al., 2014).

MATERIAL AND METHOD

A total of 10 *Rhinoceros unicornis* dung samples (G1–G10), each consisting of ~100 g, were collected from the *Rhinoceros unicornis* dung midden close to the road within the grassland area in the western part of the Kaziranga National Park. Samples were collected from the center to periphery of the dung midden. Another 10 samples (F1–F10) of similar size were collected from a dung midden in the forested area close to the road in the central part of the park. The accumulations of rhino dung in the sites sampled are the result of consistent use by multiple individuals of *Rhinoceros unicornis* for at least several years. The middens were about 27.9–32.5 m² in area and approximately 0.6 m in thickness. The dung samples were collected from above the ground level and below the surface of the dung to avoid contamination by the surface soil and atmospheric particles (Fig. 2b–d).

The dung samples were processed using the standard acetolysis method (Erdtman, 1943). Samples were successively treated with 10% aqueous potassium hydroxide (KOH) solution to deflocculate the sediments, 40% hydrofluoric acid (HF) to dissolve silica, and acetolysis (9:1 anhydrous acetic acid to concentrated sulphuric acid, [H₂SO₄]). Thereafter, the samples were treated twice with glacial acetic acid (GAA), and washed 3 or 4 times with distilled water. The samples were then transferred to a 50% glycerol solution with a few drops of phenol to protect against microbial decomposition. Excluding pollen grain and fern spores, 421 to 470 fungal spores were counted from each sample to produce the fungal spore spectra. Observation of the fungal spores and microphotographs was performed using an Olympus BX-61 microscope with DP-25 digital camera under 40x magnification (Fig. 3). The identified fungal spores were categorized as coprophilous and non-coprophilous fungi. We consulted the literature as well as published papers to aid in identification of fungal spores (van Geel, 2003; Cugny et al., 2010; Gross, 2011; Mungai et al., 2011; Basumatary et al., 2014; van Asperen et al., 2016).

RESULTS

Fungal spore spectra

The fungal spectra in *Rhinoceros unicornis* dung samples from the forested and grassland area are listed in Tables 2 and 3. In the forested area, 10 dung samples (F1–F10) collected from the rhino dung midden located in the forested area of Kaziranga National Park (Fig. 1) were characterized by the predominance of coprophilous fungi (70.7%) over non-coprophilous fungi (29.3%). Among coprophilous fungi, *Sporormiella* (18.4%) was the most common, followed by *Ascodesmis* (17.4%) and *Saccobolus* (17.2%). *Cercophora*,



Figure 2. (a) *Rhinoceros unicornis* in its natural habit in Kaziranga National Park (b) A view of a field photograph during midden dung observation by Basumatary. (c) Sampling locations (red numbers) on *Rhinoceros unicornis* midden in forest area. (d) Sampling locations (red numbers) on *Rhinoceros unicornis* midden in grassland area. (For interpretations of the references to color in this figure legend, the reader is referred to the web version of this article.)

Gelasinospora, *Podospora*, and *Sordaria* had values of 2.3% to 6.4%. The non-coprophilous fungi, chiefly the Microthyriaceae, *Glomus*, *Tetraploa*, *Meliola*, and *Helminthosporium*, were recorded within the range of 1.5% to 4.6% (Fig. 4).

The 10 dung samples (G1-G10) collected in the grassland area from the *Rhinoceros unicornis* dung midden located in the forested area of Kaziranga National Park (Fig. 1) were also characterized by the dominance of coprophilous fungi (77.9%) over non-coprophilous fungi (22.1%). Among coprophilous fungi, the *Sporormiella* (20.3%), *Ascodesmis* (18.6%), and *Saccobolus* (18.3%) are the most common taxa, while *Cercophora*, *Coniochaete*, and *Sordaria* varied from 2.3 to 7.7%. The non-coprophilous fungi are chiefly *Helminthosporium*, *Cookeina*, *Tetraploa*, and *Alternaria*, which ranged from 1.0 to 4.9% (Fig. 4).

DISCUSSION

A total of 18 fungal spore types were identified in *Rhinoceros unicornis* dung midden samples collected both from grassland and forest area samples in Kaziranga National Park. The coprophilous fungi were predominant over non-coprophilous fungi in grassland and forested areas. The study reveals that *Sporormiella*, *Saccobolus*, and *Ascodesmis* were frequent and dominant in all of the studied samples. Baker et al. (2013) listed spore types associated with megaherbivore

dung based on empirical evidence and, while they included *Sporormiella*, neither *Saccobolus* nor *Ascodesmis* were included in their study. Richardson (2001) documented that some taxa of coprophilous fungi may have a preference for specific dung types and further research is needed to confirm whether these later two taxa are specific to *Rhinoceros unicornis* dung. However, other coprophilous fungi such as *Cercophora*, *Sordaria*, *Podospora*, and *Gelasinospora* listed by Baker et al. (2013) were also consistently present in the assemblage from the *Rhinoceros unicornis* dung. It is, however, the presence of *Sporormiella* in the surface and lake soil sediments that currently is considered to serve as a powerful proxy for the present and past existence of herbivores and birds as a part of the paleoecological reconstruction of a region (van Geel et al., 2003; Graf and Chmura, 2006; Raper and Bush, 2009; Parker and Williams, 2012; Etienne et al., 2013). The presence of the spores of *Sporormiella* greater than 2% in a pollen sample is considered a strong indication of the presence of megafauna in the region (Davis, 1987; Raczka et al., 2016). Feranec et al. (2011) noted that more taphonomic study is needed on how spores of coprophilous fungi enter the stratigraphic record and to identify the preservation potential of *Sporormiella* spores in different habitat and sediment types. They cite Nyberg and Persson (2002), who show that fungal diversity in moose (*Alces alces*) dung was promoted in pine forest but suppressed in spruce forest. Our study partially addresses the issue of differential spore preservation. There does not appear to be any

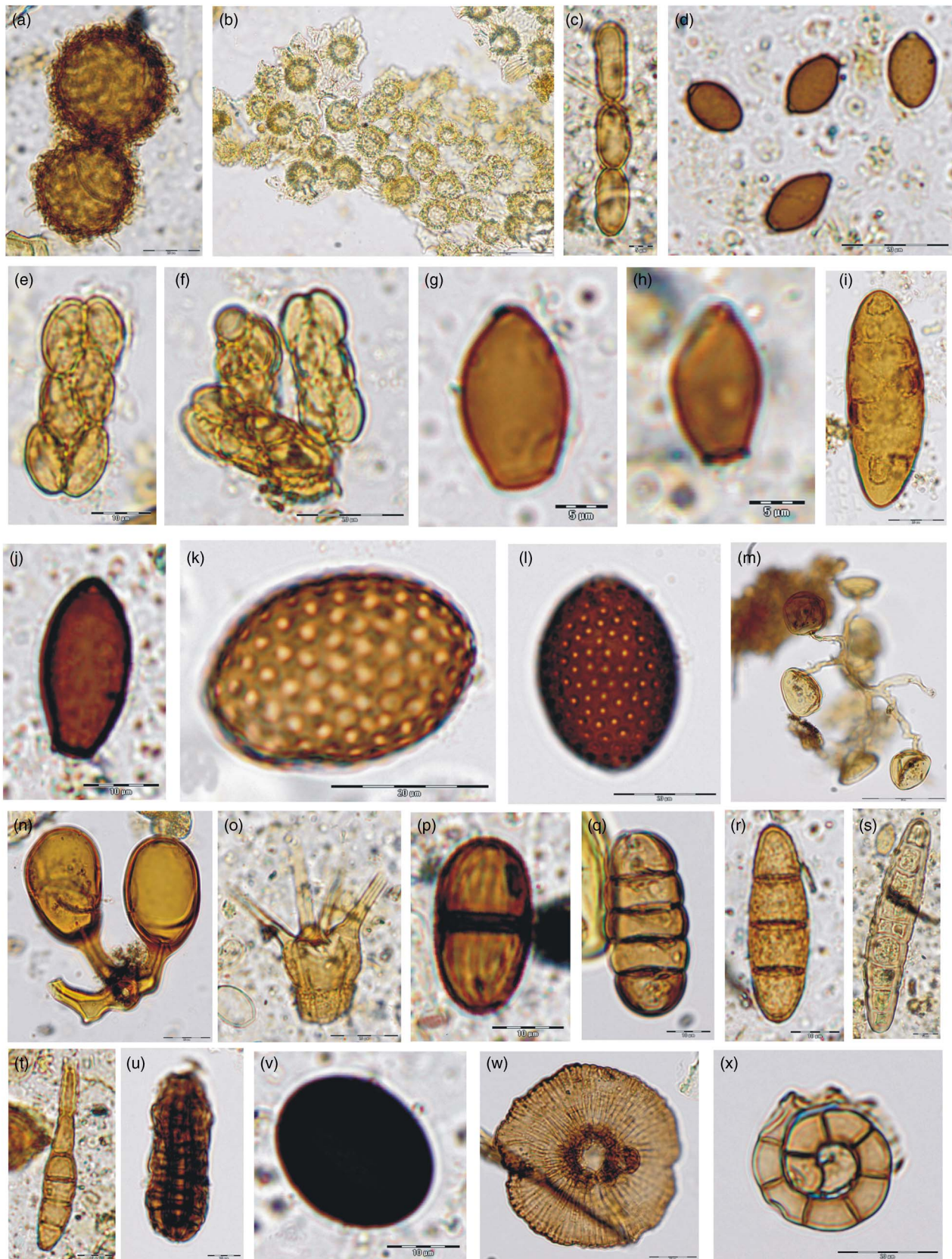


Figure 3. (color online) Fungal remains recovered from *Rhinoceros unicornis* dung midden samples in Kaziranga National Park, India. (a and b) Clumping of *Ascodesmis*. (c) Clumping of *Sporormiella*. (d) Group of *Sporormiella*. (e and f) Clumping of *Saccolobus*. (g and h) *Cercophora*. (i) *Podospora*. (j) *Sordaria*. (k and l) *Gelasinospora*. (m and n) *Glomus* with Hyphae. (o) *Tetraploa*. (p) *Cookeina*. (q and r) *Meliola*. (s) *Helminthosporium*. (t) *Alternaria*. (u) *Dictyosporium*. (v) *Nigrospora*. (w) Microthyriaceae. (x) *Helicoon*.

Table 2. The fungal spore frequency data recovered from the rhino dung midden from the forest area. Numbers are given as percentages.

Sample number	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Name of fungal taxa										
<i>Sporormiella</i>	17.7	19.3	17.3	16.6	18.5	19.4	19.7	20.2	16.8	18.1
<i>Saccobolus</i>	18.2	17.1	17.3	17.9	17.2	17.6	15.7	17.7	16.8	16.8
<i>Ascodesmis</i>	17.0	15.9	17.9	18.4	18.8	17.6	17.7	17.2	16.5	17.6
<i>Cercophora</i>	4.6	4.9	4.8	3.6	3.4	5.2	3.8	5.1	6.4	3.5
<i>Sordaria</i>	5.1	3.4	3.3	3.1	2.4	4.4	3.3	2.5	3.6	4.3
<i>Gelasinospora</i>	3.0	4.9	4.1	2.8	3.4	2.3	3.0	3.3	2.8	3.3
<i>Podospora</i>	3.0	3.4	3.8	4.6	3.7	2.1	3.5	3.0	2.3	3.0
<i>Coniochaete</i>	2.3	3.2	2.8	3.6	3.4	2.3	3.0	2.8	3.1	3.8
<i>Nigrospora</i>	2.0	2.2	1.5	1.8	1.3	1.8	1.5	1.8	2.1	1.5
<i>Glomus</i>	3.8	3.9	3.1	3.6	3.4	4.4	4.8	5.6	4.4	4.5
<i>Tetraploa</i>	4.6	4.6	5.4	4.6	4.2	3.4	4.5	4.3	4.6	4.0
<i>Helminthosporium</i>	2.5	2.2	3.1	3.6	3.2	2.6	3.5	2.3	2.3	2.5
<i>Alternaria</i>	1.5	2.0	1.5	1.8	2.6	2.8	2.0	3.0	3.9	3.3
<i>Cookeina</i>	3.8	3.4	4.6	4.3	3.4	3.1	3.5	2.5	2.3	2.0
Microthyriaceae	3.5	2.9	2.8	2.6	3.2	3.6	3.3	2.5	4.1	3.5
<i>Meliola</i>	3.0	2.4	2.0	2.8	3.7	2.6	3.0	2.5	2.8	3.3
<i>Dictyosporium</i>	2.0	2.2	2.6	2.0	2.4	2.6	2.0	2.3	2.3	2.0
<i>Helicoon</i>	2.3	2.0	2.0	2.3	1.6	2.3	2.0	1.5	2.8	3.0

difference in the relative abundance of fungal spores in the dung of *Rhinoceros unicornis* recovered from two distinct habitats, forest and grassland. Similarly, the presence of *Sporormiella* in Pleistocene samples have been used as a direct indicator for the presence of extinct megaherbivores based on the study of mammoth (*Mammuthus columbi*) dung recovered in Bechan Cave (southern Utah) (Davis, 1987). The clumping of coprophilous fungi spores, especially *Sporormiella*, *Saccobolus*, and *Ascodesmis*, in the *Rhinoceros unicornis* dung midden samples was very common and indicative of their local origin. In coprophilous fungi, especially

Sporormiella, the spores have low dispersal capacity and produce localized concentrations rather than being dispersed across the region and generally are transported less than 100 m from the source area (Davis and Shafer, 2006; Raper and Bush, 2009; Parker and Williams, 2012; Gill et al., 2013). The fungal spores are therefore strictly local in origin and become preserved close to the source where sporulation occurred (van Geel and Aptroot, 2006). In our study of *Rhinoceros unicornis* dung midden samples, the *Sporormiella*–*Saccobolus*–*Ascodesmis* assemblage was a strong indicator of rhinoceros, as indicated by their regular presence and high abundance in all the samples.

Table 3. The fungal spore frequency data recovered from the rhino dung midden from the grassland.

Sample number	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Name of fungal taxa										
<i>Sporormiella</i>	21.3	20.9	18.9	18.3	20.5	20.1	21.6	21.4	19.9	20.0
<i>Saccobolus</i>	19.5	18.6	18.4	17.0	19.7	19.3	17.8	18.4	19.1	18.3
<i>Ascodesmis</i>	18.2	15.8	18.6	17.3	16.8	20.1	19.8	18.2	18.6	19.5
<i>Cercophora</i>	5.2	5.8	5.5	5.0	5.6	6.0	4.6	5.8	7.7	4.9
<i>Sordaria</i>	3.9	4.7	4.3	3.7	2.7	4.5	3.8	2.9	4.3	4.7
<i>Gelasinospora</i>	3.6	5.8	5.0	4.2	4.5	3.0	3.6	3.9	3.2	3.5
<i>Podospora</i>	3.9	4.4	4.3	5.2	4.3	2.8	4.1	3.2	2.9	3.5
<i>Coniochaete</i>	2.6	3.3	3.3	4.5	4.0	2.5	2.3	2.9	3.5	4.2
<i>Nigrospora</i>	1.3	1.4	1.0	1.6	1.1	1.5	1.0	1.0	1.6	2.2
<i>Glomus</i>	3.4	3.5	2.5	3.4	2.9	4.0	4.6	4.9	3.7	4.2
<i>Tetraploa</i>	3.6	2.8	3.8	4.5	3.5	2.5	3.8	3.2	2.9	3.5
<i>Helminthosporium</i>	3.9	3.0	4.5	5.2	4.3	3.5	4.6	2.7	2.7	2.7
<i>Alternaria</i>	1.3	1.4	1.8	2.4	1.3	2.0	1.5	1.7	1.3	2.2
<i>Cookeina</i>	2.6	2.1	2.0	1.8	2.1	1.5	1.0	1.5	1.3	1.0
Microthyriaceae	1.8	1.4	1.3	2.1	1.6	1.8	2.3	2.4	2.1	1.5
<i>Meliola</i>	1.6	2.1	1.0	1.6	2.1	2.5	1.3	1.9	1.9	1.2
<i>Dictyosporium</i>	1.3	1.4	1.8	1.0	1.3	1.5	1.0	1.7	1.3	1.7
<i>Helicoon</i>	1.0	1.6	2.0	1.3	1.6	1.0	1.3	2.4	1.9	1.2

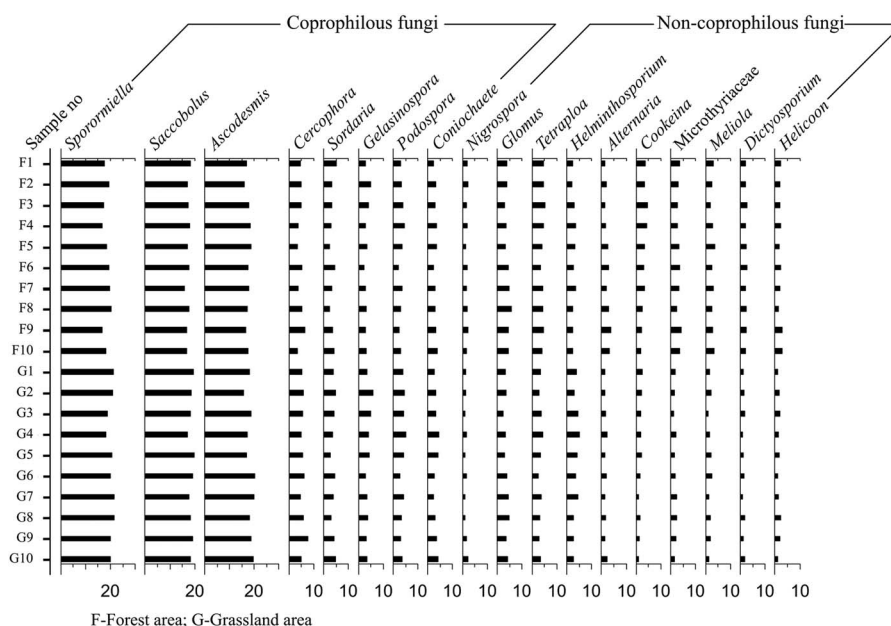


Figure 4. Comparison of fungal spectra of *Rhinoceros unicornis* dung midden samples collected from the forest and grassland area in Kaziranga National Park, India.

The high amount of coprophilous fungi along with other associated fungal spores is indicative of the warm and humid condition in the region because water availability is the important factor for the germination and sporulation of *Sporormiella* (Austin, 1958; Ingold and Marshall, 1962; Kuthubutheen and Webster, 1986a, 1986b). The presence of *Cercophora* in the assemblage has also been observed in dung and is also an indicator of woodland and grassland environments (Blackford and Innes, 2006; Graf and Chmura, 2006). The *Sporormiella*–*Saccobolus*–*Ascodesmis* assemblage is considered specifically characteristic for *Rhinoceros unicornis* dung based on their abundance in the dung midden samples. In our dataset, it is observed that, in addition to the *Sporormiella*, the other two taxa, *Saccobolus*, and *Ascodesmis*, were most frequently present with values >15%. The *Sporormiella*–*Saccobolus*–*Ascodesmis* assemblage is characteristic of rhino dung, as indicated by their consistent high frequency (16–21%) and clustering spores in all the examined samples. In contrast, non-coprophilous fungi such as *Tetraploa*, *Cookeina*, *Meliola*, and *Dictyosporium* have a relatively low presence in the assemblage and the consistency of their presence in the dung needs more investigation. The high body temperature (~37°C) and acidic environment in the gut of herbivorous animals may be the main reason that non-coprophilous fungal spores are destroyed if they are consumed during feeding.

The continuous and comparatively high presence of Microthyriaceae (epiphyllous fungi) and *Helicoon* in the dung samples collected from the forest area was very significant (1–2%). The presence of such fungi in the assemblage is indicative of the dense forest vegetation under warm and humid conditions in response to the high rainfall in the region (Cookson, 1947; Selkirk, 1975; Reddy et al., 1982;

Johnson and Sutton, 2000; Limaye et al., 2007; Hofmann, 2010; Medeanic and Silva, 2010). The comparatively high value of *Helminthosporium* (3.7%) in the assemblage of grassland dung samples is of interest, as it is a common pathogen in grasses. The presence of *Tetraploa* and *Glomus* in the assemblage of both area samples suggests water-logged conditions with rich plant diversity that might be incorporated through the ingestion of plants, water, and soil, as these fungi are commonly found on leaf bases, roots, and stems of Poaceae and Cyperaceae (Ellis, 1971; Tanaka et al., 2009).

CONCLUSIONS

This study demonstrates that the *Sporormiella*–*Saccobolus*–*Ascodesmis* assemblage is distinctive and characteristic of *Rhinoceros unicornis* dung. The documentation of the coprophilous fungi present in surface soil sediments in the region can complement the data provided by the analysis of the *Rhinoceros unicornis* dung midden samples in Kaziranga National Park and its vicinity. The resulting fungal dataset on *Rhinoceros unicornis* dung can provide a baseline that can help us to document the past presence of *Rhinoceros unicornis* based on the study of sedimentary soil profile in Kaziranga National Park and neighboring regions. The dataset also can serve as a powerful tool to determine the past distribution and ecology of *Rhinoceros unicornis* in India and neighboring areas when other evidence such as bones are not available. Combined with a study of the pollen and fungal spores preserved in the dung of other herbivores animals in the region, this approach provides a way of recognizing the distribution of other animals that are found in the same habitat as *Rhinoceros unicornis* prior to their extirpation. Further research is needed that includes surface

and sedimentary soil samples beyond the perimeter of the *Rhinoceros unicornis* dung midden to determine if they preserve a different or similar fungal spore assemblage than that seen in the *Rhinoceros unicornis* dung midden samples.

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