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Pollen and non-pollen palynomorph preservation in the dung of the Greater One-horned Rhino (*Rhinoceros unicornis*), and its implication to palaeoecology and palaeodietary analysis: A case study from India

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ABSTRACT

The Greater One-horned Rhino of India is an endangered species. We investigate the pollen and non-pollen palynomorphs preserved in rhino dung collected from a communal rhino dung midden in Kaziranga National Park to document the vegetation composition and dietary habits of this rhino. The palynodata reflects the dominance of nonarboreals over arboreals from forested and grassland areas respectively. The arboreals include a mixture of evergreen, riparian, and deciduous taxa, which are strongly indicative of different types of forest in the park. The high presence of grass pollen and phytoliths in the rhino dung was marked and confirmed that grass is the primary food of the rhino. Diatoms and Thecamoeba in the rhino dung assemblage was suggestive of the ingestion of huge amount of water and the water logged condition in parts of the park. Energy dispersive spectroscopy analysis indicated that the silica contained in rhino dung sample was high in comparison to the others elements and determination of its presence is useful for understanding rhinoceros' habits. The generated data will be helpful and to serve as a guideline for subsequent palaeoecological and palaeodietary studies in the park.

1. Introduction

The Greater One-horned Rhino (*Rhinoceros unicornis*, Linnaeus, 1758) is one of the largest herbivorous wild animals in the world. This rhinoceros is completely herbivorous and consumes a significant quantity of plants each day including terrestrial, marshy and aquatic species. One of the unique behaviors of rhinos, including the Greater One-horned Rhino is to consistently use the same location for their daily excretion and dung may be deposited at this site or midden by multiple individuals for up to several years (Dinerstein, 2003; pers. comm. local community). The systematic study of the modern pollen and vegetation relationship in Kaziranga National Park and wildlife sanctuary is difficult due to the high risk of disturbance to the wildlife and the ecology. Therefore, the study of pollen and non-pollen palynomorphs from the rhino dung has the potential to examine the relationship between the modern pollen and vegetation and the diet of the rhino with minimal impact to the animal or local vegetation. Previous work on the relationship between modern pollen and vegetation based on animal dung has shown it serves as one of the best analogues of local and regional vegetation (Moe, 1983; Carrion, 2002; Kropf et al., 2007). Similarly, in some habitats such as deserts and other regions such as swamp and wetlands as well as areas where animal dung is commonly encountered, the dung serves as a substrate that can be useful for the preservation of pollen.

The study of coprolites as a substrate that preserves pollen can facilitate the study of the palaeodiet of wildlife in relation to the palaeovegetation and climate in a region (Wood et al., 2013). Studies of coprolite palynology, especially in North America, have been used to interpret the prehistoric diet of both people and animals and the local vegetation (Martin and Sharrock, 1964; Bryant and Larson, 1968; Bryant, 1969, 1974; Riskind, 1970; Hall, 1972; Schoenwetter, 1974). However, including both phytoliths and diatoms in a single study can also serve as a powerful proxy for palaeoenvironmental reconstruction and recognizing domestic herbivores (Lu and Liu, 2003; Gallego and Distelm, 2004; Blinnikov, 2005; Boyd, 2005; Lu et al., 2005; Rovner, 1986; Bowdery, 1999; Osterrieth et al., 2009; Morris et al., 2009a, 2009b; Gross, 2011; Blinnikov et al., 2013). There are few studies that have examined both palynomorphs and phytoliths in coprolites (Bryant, 1974; Bryant and Williams-Dean, 1975; Horrocks et al., 2002, 2003; Ghosh et al., 2008; Scott et al., 2016). This is the first study of the palynomorphs combined with Field Emission Scanning Electron Microscope with Energy Dispersive Spectroscopy (FESEM-EDS) analysis in rhino dung to observe the vegetation composition in

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relation to the preference of dietary plants and climate in the habitat of the Greater horned rhinoceros. Based on these studies the identification of the palynomorphs preserved in the dung midden of the rhino serve as a baseline that can be used as a proxy for palaeoecological and palaeodiatary reconstruction of past distribution of rhino.

2. Study site, vegetation and climate

The Kaziranga National Park of India is located between the latitudes of 26°32′ N and 26°47′ N, and longitudes 93°07′ E to 93°38′ E, and the elevation ranges between 45 and 90 m a.s.l., and serves as an ideal site to study the dietary habits and ecology of the Greater One-horned Rhino (Figs. 1, 2a,b). In general, there are mainly five different types of vegetation composition in the park; tropical, semi-evergreen, deciduous, savannah, and grassland (Champion and Seth, 1968) (Fig. 2c,d). Alluvial grassland is dominant in the park covering 50.6%, followed by woodland 21.8%, short grassland 7.7% and eroded land occupies 11.7% in the national park (Das et al., 2014). The dominant tree taxa include; *Albizia lebbeck*, *Acacia catechu*, *Mesua ferrea*, *Cinnamomum bijolghota*, *Magnolia hodgsonii*, *Aphanamixis polystachya*, *Albizia procera*, *Dillenia indica*, *Salmalia malabaricum*, *Terminalia billirica*, *Terminalia myriocarpa*, *Syzygium cumuni*, *Duabanga grandiflora*, *Lagerstroemia speciosa*, *Grewia serrulata*, and *Ficus glomerata*. In the grassland, the dominant tall grass is mainly *Erianthus ravennae*, *Phragmites karka*, *Arundo donax*, *Imperata cylindrica*, and *Saccharum procerum*. The ferns are dominated by *Lycopodium clavatum*, *Dryopteris filix-mas*, *Gleichenia dichotoma*, *Adiantum caudatum*, *Drynaria rigidula*, *Lygodium japonicum*, *Polypodium microrhizoma*, *Diplazium esculatum*, and *Blechnum occidentale*. The marshy taxa include *Polygonum orientale*, *Cyperus rotundus*, and *Sagittaria sagittifolia*, and the aquatic taxa are dominated by *Eichhornia crassipes*, *Trapa bispinosa*, *Azolla pinnata*, *Potamogeton pectinatus*, *Vallisneria spiralis*, *Nymphaea nouchali*, *Nymphaea alba*, *Euryale ferox*, *Myriophyllum indicum*, *Nymphoides indica*, and *Nymphoides cristatum*, all of which are common in the swamp within the national park. The climate of the region is controlled by the southwest and northeast monsoons, it is hot and humid during summer and cold and dry during winter. During summer the maximum temperature ranges from up to 37 °C to a minimum of 4 °C during winter. 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 national park. The soil composition varies from site to site and includes sandy loam soil in forest, sandy soil in grassland, and clayey in the swamp and water bodies.

The greater one horned rhino generally prefers alluvial flood plain habitat with grassland, along with scattered woodlands and swamps. Many studies have been carried out on the rhinoceros habit and diet and have documented that the grasses are the primary food (70–87%), followed by woodland and aquatic plant species (Brahmachary et al., 1971; Laurie, 1978, 1982; Hazarika and Saikia, 2012). However, it must be noted that the incorporation of the palynomorphs (pollen grains, ferns, and phytoliths) in the dung is secondary and are consumed during eating of other plants, soil from the forest floor and swamp sediments. The occurrence of diatoms and Thecamoeba are the result of direct ingestion of water. Wind and flood water are also

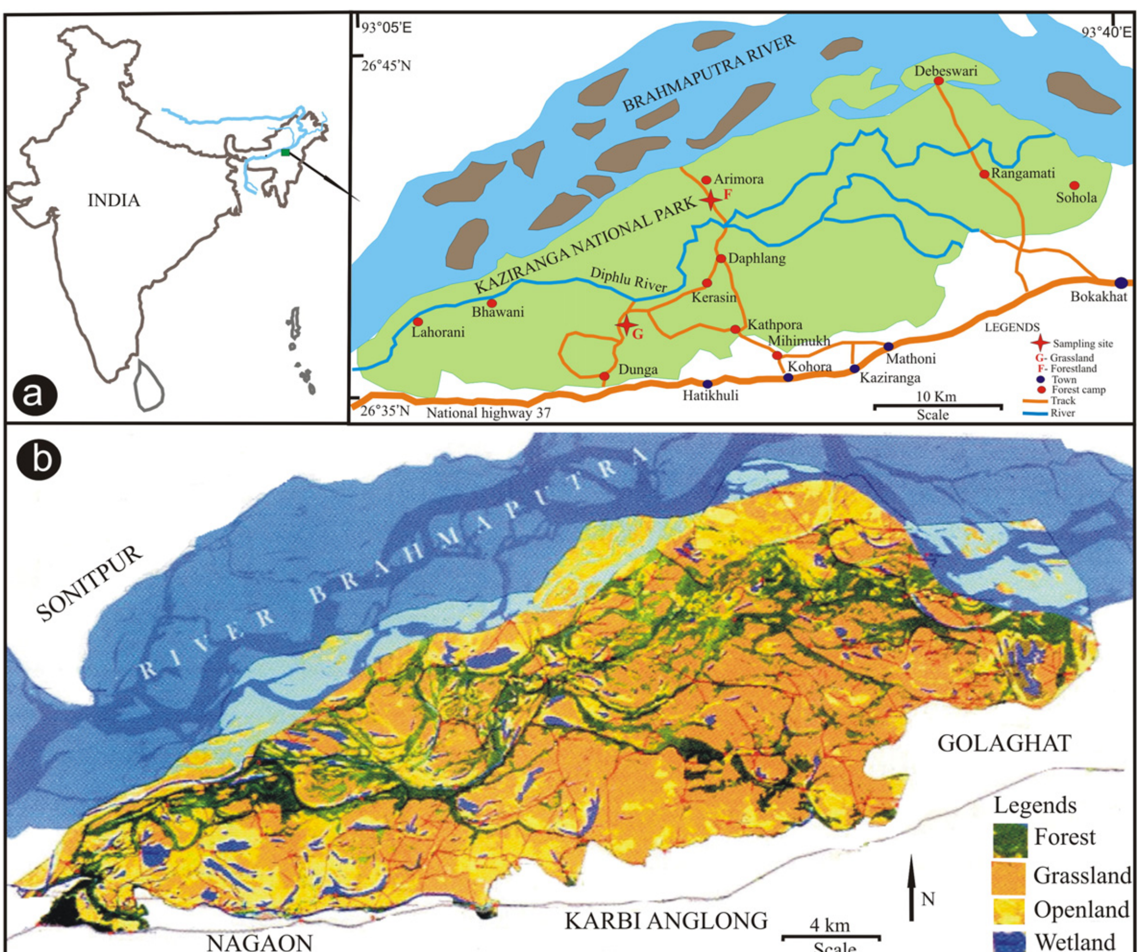

Fig. 1. (a). Location map showing the study sites. (b). Satellite and Land cover map of the Kaziranga National Park, India. Source, Das et al., 2014.

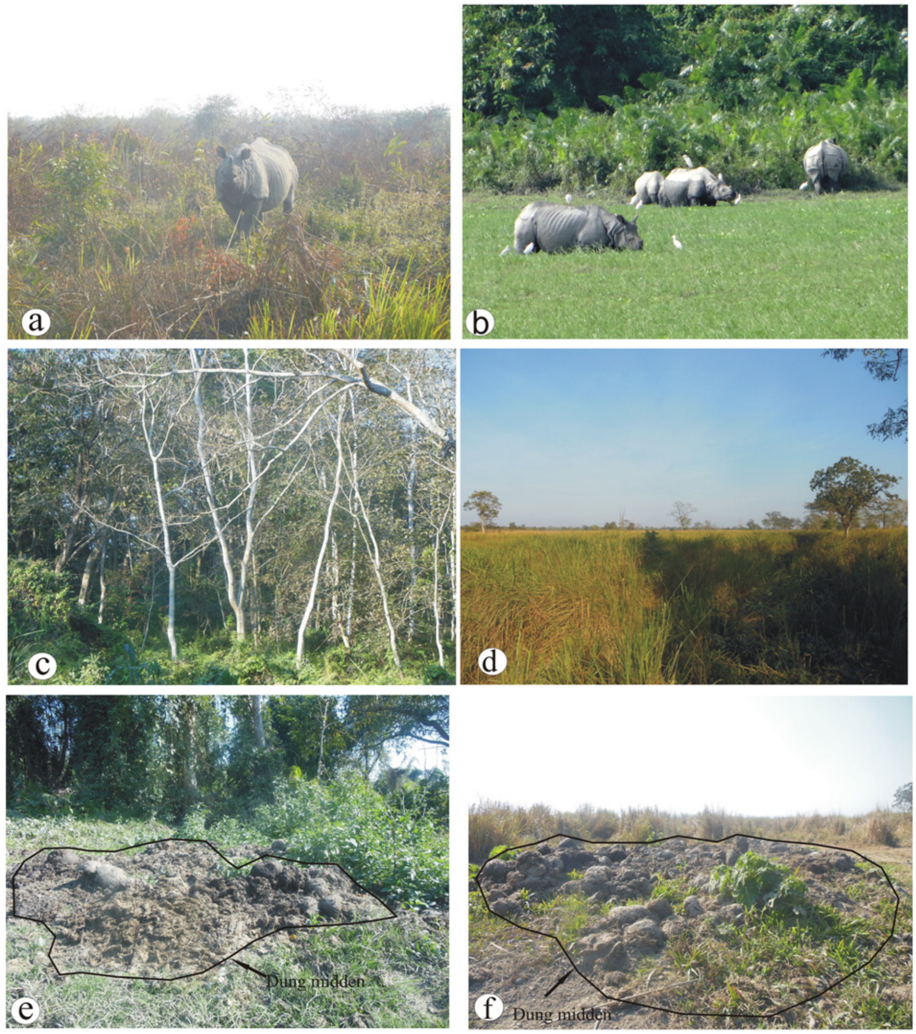

Fig. 2. Field photographs from the Kaziranga National Park.
(a) View of a rhino in natural habit
(b) A view of the rhinos during feeding time
(c) Evergreen forest in Kaziranga National Park
(d) View of grassland in Kaziranga National Park
(e) A view of midden rhino dung in forested area.
(f) A view of midden rhino dung in grassland area.

agents transport of the palynomorphs from higher elevations that are subsequently incorporated in the rhino dung.

3. Materials and methods

3.1. Sampling

During the month of January 2015, the first author visited the site and a total of ten rhino dung samples, each consisting of approximately 100 g, were collected randomly at 1 m intervals from the rhino dung midden (Fig. 2e,f) close to the road side within the grassland area from the western part of the national park. Similarly, another ten samples of similar size were collected from a dung midden in the forested area close to the road from the central part of the national park. It was expected that the accumulation of rhino dung in the sampling location was the result of consistent use by multiple rhinos for at least several years and the middens were about 300–350 ft^2 and approximately 2 ft 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.

3.2. Laboratory methods

3.2.1. Sample analysis

The palynological samples were processed using the standard acetolysis method (Erdtman, 1953). Samples were successively treated with 10% aqueous KOH solution to deflocculate from the sediments, 40% HF to dissolve silica, and acetolysis (9:1 anhydrous acetic acid to concentrated (H_2SO_4). Samples were transferred to a 50% glycerol solution with a drop of phenol. The fungal spores, algal remains, and thecamoeba were observed on the same palynological slide. A total of 204 to 276 palynomorphs; including pollen, fungal remains, ferns, algal remains and Thecamoeba were counted from each sample to create the palynomorph spectra. The recovered taxa were categorized as arboreal taxa (tree and shrub), nonarboreal taxa (marshy, aquatic and terrestrial herb), upland taxa, ferns, fungal remains, algal remains, and Thecamoeba.

For the diatom analysis, the samples were treated with concentrated hydrochloric acid (HCL) to dissolve carbonates and then treated with a mixture of hot nitric acid (HNO_3) and potassium dichromate to dissolve organic materials. The samples were washed with distilled water 2 to 4 times and permanently mounted on a slide with Canada balsam for microscopic observation. Similarly, the phytoliths were observed on the same diatom slide because of the availability and clarity in the assemblage. The total number of diatoms and phytoliths (202 to 264) was counted from each sample to create the diatom and phytolith spectra. For the identification of modern plynomorphs, we consulted the reference slides in the Birbal Sahni Institute of Palaeosciences (BSIP) herbarium of Lucknow (India) as well as published papers and photographs (West and West, 1903; Chauhan and Bera, 1990; Bera et al., 2009; Barboni et al., 2007; Cugny et al., 2010; Gross, 2011; Blinnikov et al., 2013; Farooqui et al., 2013; Basumatary et al., 2014). Observation and microphotographs were done using both an Olympus BX-61 microscope with DP-25 digital camera under 40X magnification and Field Emission Scanning Electron Microscope (FESEM) (Figs. 3, 4, 5). The Field Emission Scanning Electron Microscope (FESEM) with Energy Dispersive Spectroscopy (EDS) analysis was also performed using FESEM (JEOL, JSM-7610F) equipped with EDS (EDAX, USA instrument) operated at 25 keV to determine the elemental composition of the rhino dung.

4. Results

The palynomorphs spectra in the rhino dung samples from the forested and grassland area are as described below.

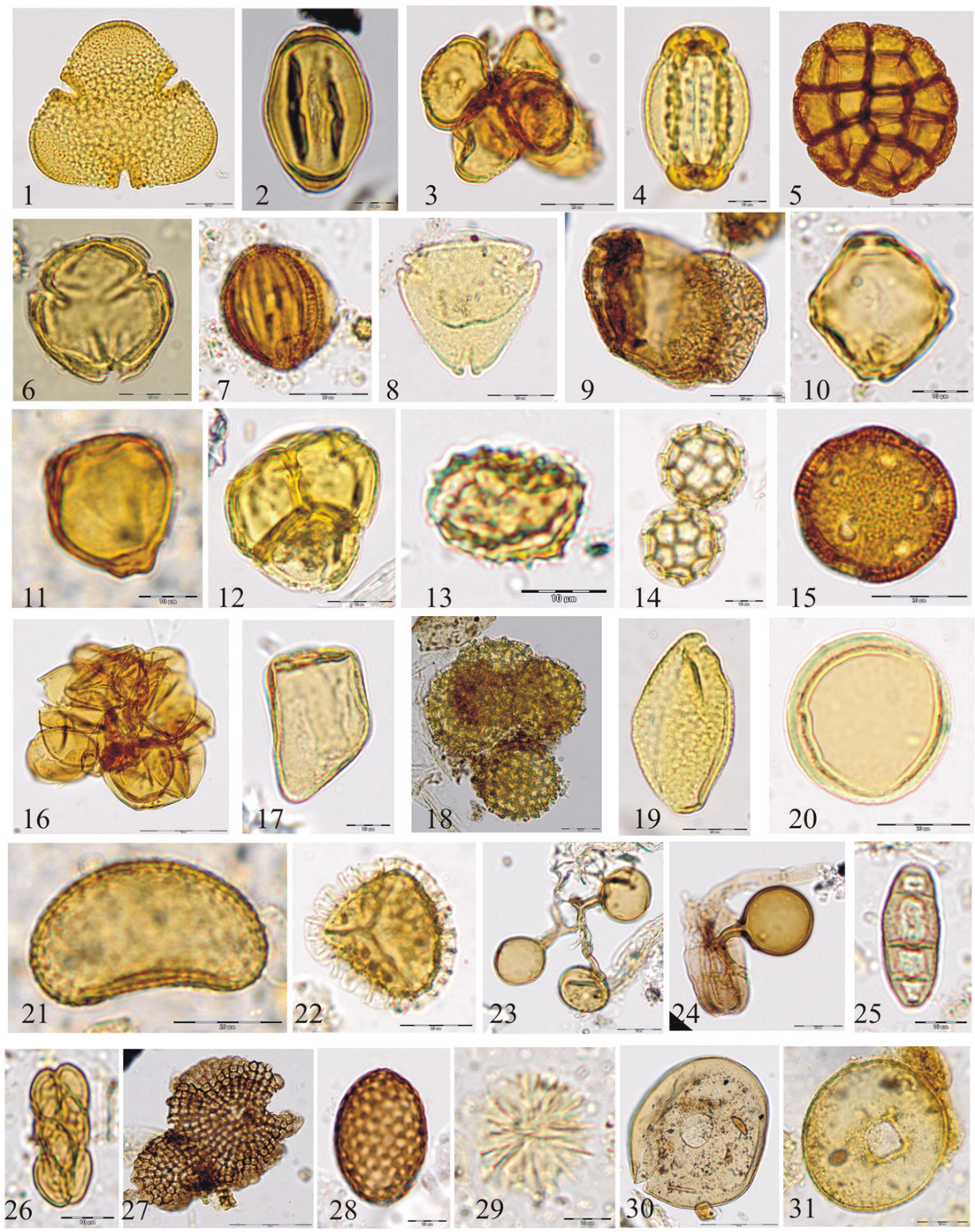

Fig. 3. Palynomorphs recovered from the rhino dung in Kaziranga National Park.

Explanation of palynomorphs

1. *Salmalia*, 2. *Lagerstroemia*, 3. *Duabanga* pollen in clumping, 4. *Barringtonia*, 5. *Acacia*, 6. *Mesua*, 7. *Strobilenthes*, 8. *Symplocos*, 9. *Pinus*, 10. *Alnus*, 11. *Betula*, 12. *Rhododendron*, 13. Tubuliflorae, 14. Liguliflorae pollen in clumping, 15. Amaranthaceae, 16. Poaceae pollen in clumping, 17. Cyperaceae, 18. *Polygonum* pollen in clumping, 19. *Eichhornia*, 20. *Nymphaea*, 21. *Dryopteris*, 22. *Lycopodium*, 23. *Glomus* with hyphae, 24. *Tetraploa* associated with *Glomus*, 25. *Helminthosporium*, 26. *Saccobolus*, 27. Microthyriaceae, 28. *Gelasinospora*, 29. *Botryococcus*, 30. & 31. *Arcella*

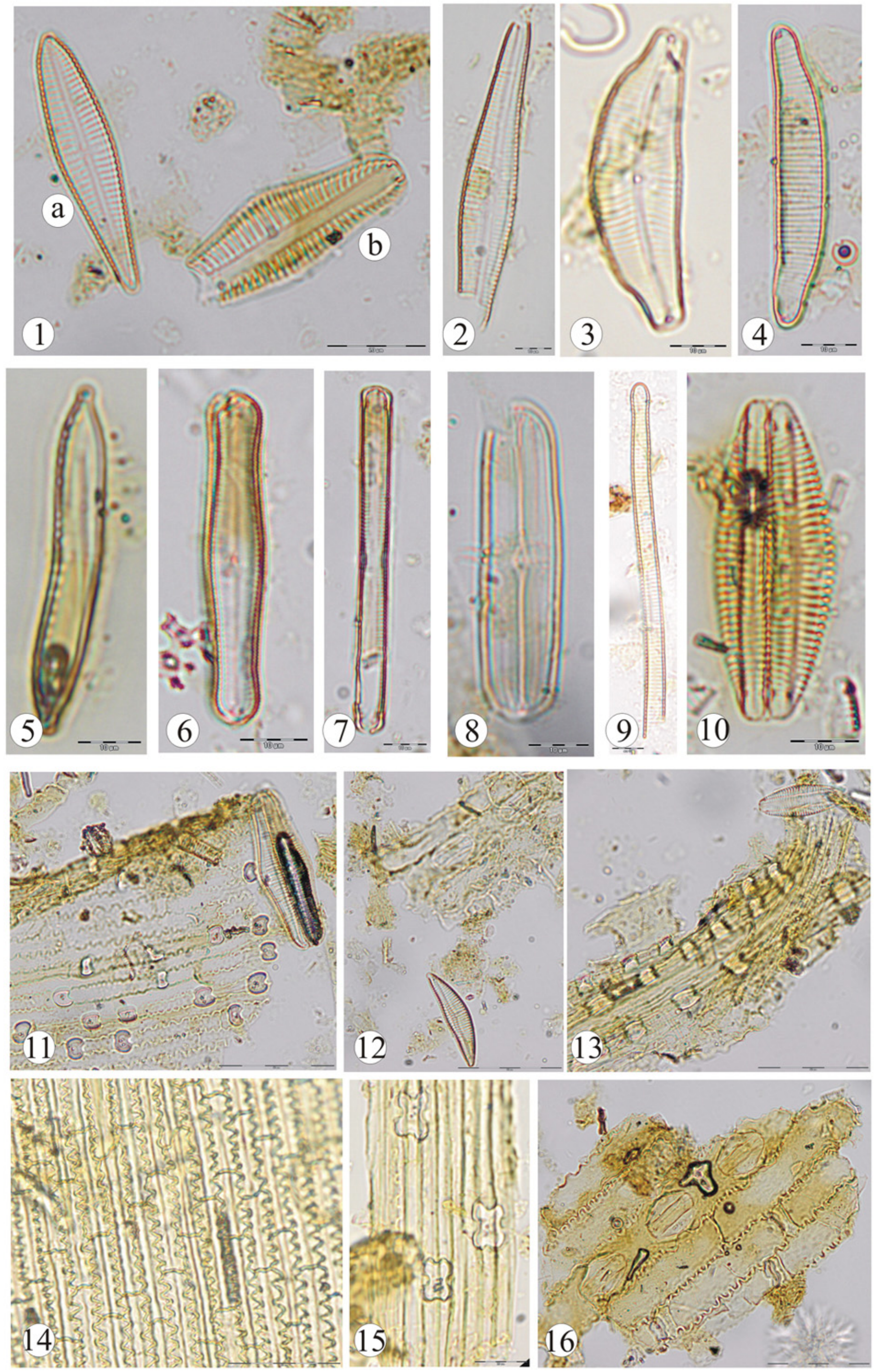

Fig. 4. Diatoms and phytoliths assemblage recovered from the rhino dung in Kaziranga National Park.

Explanation of diatoms and phytoliths

1. a. *Navicula*, b. *Rhopaloda*, 2. *Cymbella*, 3. *Cymbella*, 4. *Eunotia*, 5. *Nitzchia*, 6. *Pinnularia*, 7. *Pinnularia*, 8. *Pinnularia*, 9. *Synedra*, 10. *Navicula*, 11.12. & 13. Grass phytoliths with diatom assemblage, 14.15. & 16. C_4 grass phytoliths.

4.1. Forested area

The ten samples (F1–F10) collected from the forested area characterized by the dominance by nonarboreals (62.8%), over arboreals (16.2%). The ferns, chiefly *Dryopteris*, *Lycopodium*, and *Lygodium* comprised 7.6%, of the sample and the fungal remains; *Sporormiella*, *Cercophora*, *Saccobolus*, *Tetraploa*, and *Helminthosporium* were 7.0%. The algal remains and Thecamoeba, primarily *Botryococcus*, *Arcella*, and *Cyntropyxis*, were 1.3% and 2.9% respectively. Among nonarboreal taxa, Poaceae was dominant (27.7%). The other associated terrestrial nonarboreal taxa; Tubuliflorae, Amaranthaceae, and *Impatiens* were recorded at the values up to 4.4%. The major marshy and aquatic taxa such as Cyperaceae, Onagraceae, *Xanthium*, *Potamogeton*, *Myriophyllum*, and *Nymphaea* were consistently represented within the ranges of 0.5% to 9.7%.The arboreal taxa, chiefly *Mesua*, *Schima*, *Duabanga*, *Symplocos*, and *Strobilenthes* are present up to 3.7%. The upland taxa; *Pinus*, *Alnus*, and *Rhododendron*, were also represented at values up to 2.2%. (Fig. 6).

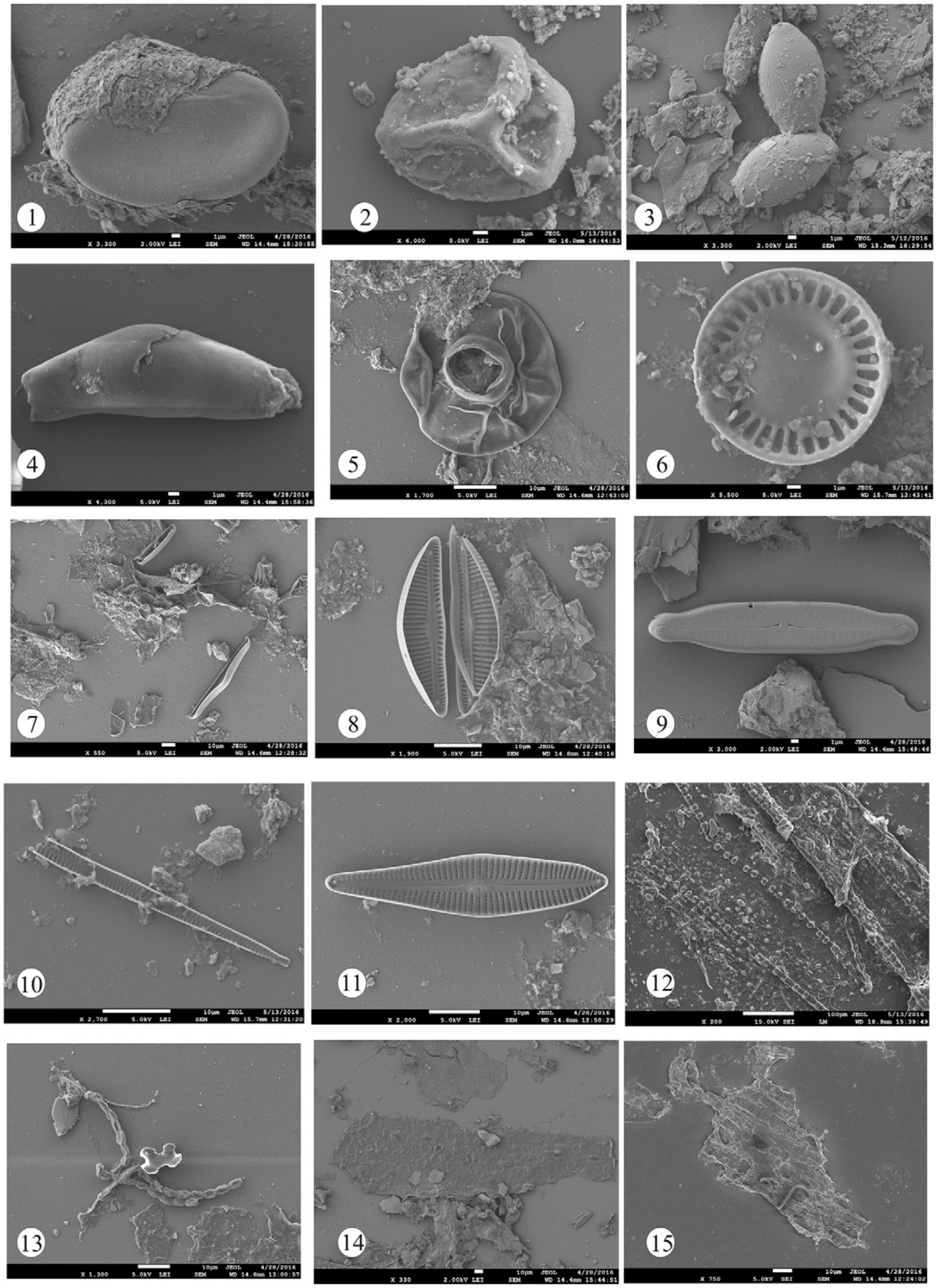

Fig. 5. Microphotographs in Field Emission Scanning electron microscopy (FESEM) in rhino dung samples collected from forested and grassland area in Kaziranga National Park.
Explanation of palynomorphs
1. Monolete, 2. Trilete, 3. *Cercophora* in clumping, 4. Fungal spore, 5. Thecamoeba, 6. *Cyclotella*, 7. Diatom assemblage, 8. *Navicula*, 9. *Pinularia*, 10. *Pinularia*, 11. *Navicula*, 12–15. C_4 grass phytoliths.

The diatoms and phytoliths in ten samples (F1–F10) collected from the forested area were also observed and characterized at 22.8% and 69.4% respectively. Diatoms, chiefly *Synedra*, *Cyclotella*, and *Navicula*, exhibited a range of 1.4% to 4.2%. Grass phytoliths, especially C_4 grass, was dominant (58.6%) and the other grass phytoliths, mainly C_3 grass (5.8%) along with dicotyledonous phytoliths (4.9%), are also consistently present in the assemblage (Fig. 7).

4.2. Grassland area

The ten samples (G1–G10) collected from the grassland habitat were characterized by the dominance of nonarboreals (75.2%), over arboreals (7.7%). The ferns and fungal remains, which include *Dryopteris*, *Polypodium*, *Saccobolus*, *Sordaria*, *Glomus*, and *Helminthosporium*, were 3.7% and 4.3% respectively. The algal remains and Thecamoeba were

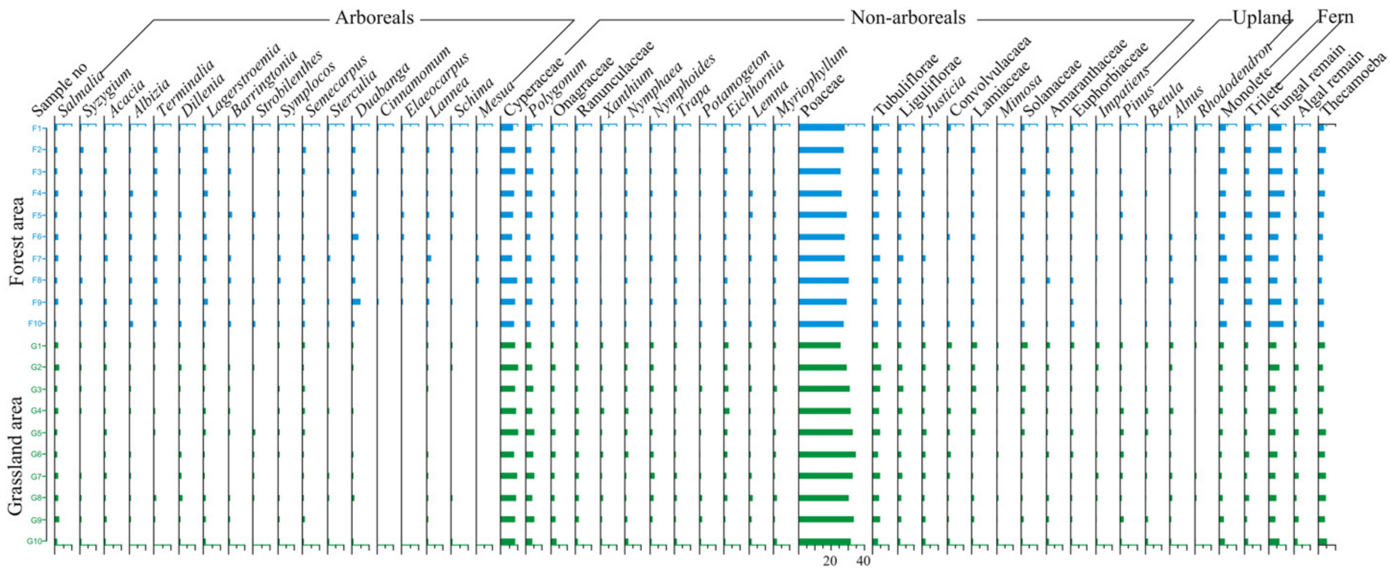

Fig. 6. Comparative palynomorph spectra from the forested and grassland sites of Kaziranga National Park.

2.0% and 3.4% respectively. Among nonarboreal taxa, Poaceae was dominant (31.1%). The other associated terrestrial nonarboreal taxa; Tubuliflorae, Liguliflorae, Convolvulaceae, and Lamiaceae were present up to 4.7%. The major marshy and aquatic taxa; Cyperaceae, *Polygonum*, *Nymphaea*, and *Eichhornia*, were represented consistently with values of 0.8% to 10%. The upland taxa; *Pinus*, *Betula*, and *Rhododendron* were only present in trace values in the assemblage. (Fig. 6).

The diatom and phytoliths in ten samples (G1–G10) collected from the grassland habitat were 16.3% and 79.3% respectively. Among diatoms, chiefly *Synedra*, *Cyclotella*, *Cymbella*, and *Navicula* the values ranged from 0.8% to 5.3%. For the grass phytoliths, C_4 grass phytoliths was dominant (68.0%) compared with the C_3 grass phytoliths (9.0%) (Fig. 7).

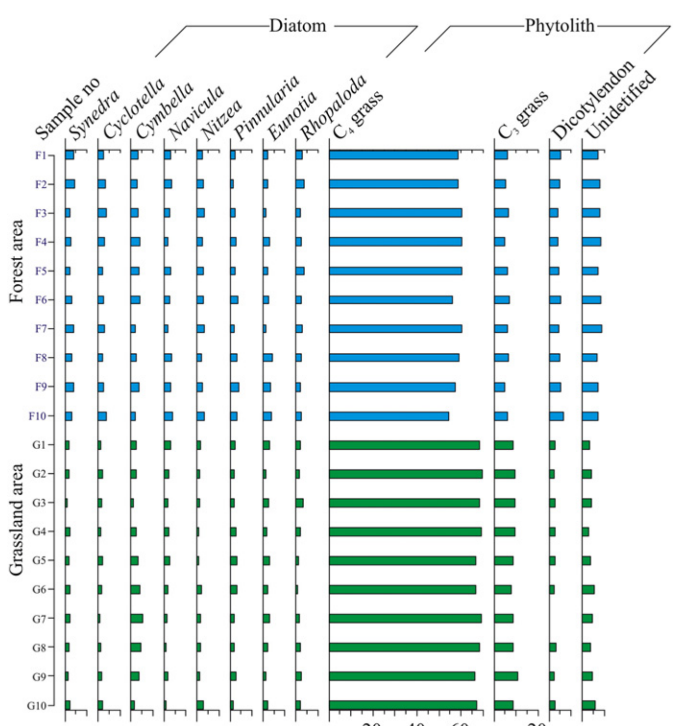

Fig. 7. Comparative diatom and phytoliths spectra from forested and grassland sites of Kaziranga National Park.

The data generated from the FESEM-EDS elemental analysis of the rhino dung samples from the forested and grassland areas are shown in Fig. 8. The rhino dung studied from the forested area showed that the O_2 level is 64.58 (weight %) followed by Si, 18.67 (weight %), K, 7.65 (weight %), Ca, 3.92 (weight %), Zr, 3.28 (weight %) and Al, 1.90 (weight %). Similarly, in the grassland area, the O_2 level is 55.71 (weight %) followed by Si, 29.41 (weight %), K, 6.92 (weight %), Zr, 3.95 (weight %), Ca, 2.62 (weight %), and Br, 1.39 (weight %) (Tables 1, 2).

5. Discussion

In general, the palynodata of the rhino dung samples from both the forested and grassland areas reflected the different types of vegetation composition, mainly tropical evergreen forest with scattered deciduous, riparian, grassland, savannah and swamp forest as indicated by the presence of evergreen, deciduous, and grass pollen which exactly reflected the current vegetation pattern in the national park. The presence of arboreal taxa; *Salmalia*, *Dillenia*, *Symplocos*, *Syzygium*, *Ficus*, *Acacia*, and *Elaeocarpus* in the assemblage, is significant and these taxa have been secondarily incorporated in the dung as the pollen adhered to items in the diet, such as the leaf, twig and flower or were deposited on the swamp or forest floor soil around the vicinity of the feeding area and ingested since rhinos are geophagous (Hazarika and Saikia, 2010). The presence of evergreen elements, mainly *Mesua*, *Schima*, and *Cinnamomum*, in the forested samples is strongly indicative of the evergreen forest that reflects the high rainfall in and around the region. The samples collected from the forested area had comparatively higher values of arboreal pollen taxa than the samples collected from the grassland area. The pollen clumping especially in grasses, *Duabanga*, Tubuliflorae, Liguliflorae, and *Polygonum*, was observed in both forested and grassland assemblage. Pollen clumping is a characteristic feature of entemophilous plants and the pollen disperses shorter distances than solitary pollen grains (Faegri and Van Der Pijl, 1966; Martin et al., 2009). However, in our study the direct ingestion of plants with flower buds by the rhinoceros in the region might also account for the occurrences of pollen clumping in the palynomorphs assemblage. However, the presence of *Lagerstroemia*, *Barringtonia*, and *Duabanga*, is indicative of the riparian forest in the region which is a very important part of the rhino habit.

The recovery of upland taxa, chiefly *Pinus*, *Betula*, and *Alnus*, in the assemblage is strongly indicative of wind activity in the region which can deposit their pollen on plants and the soil ingested by the rhinoceros. While *Rhododendron* pollen is present, it is only as trace amounts in

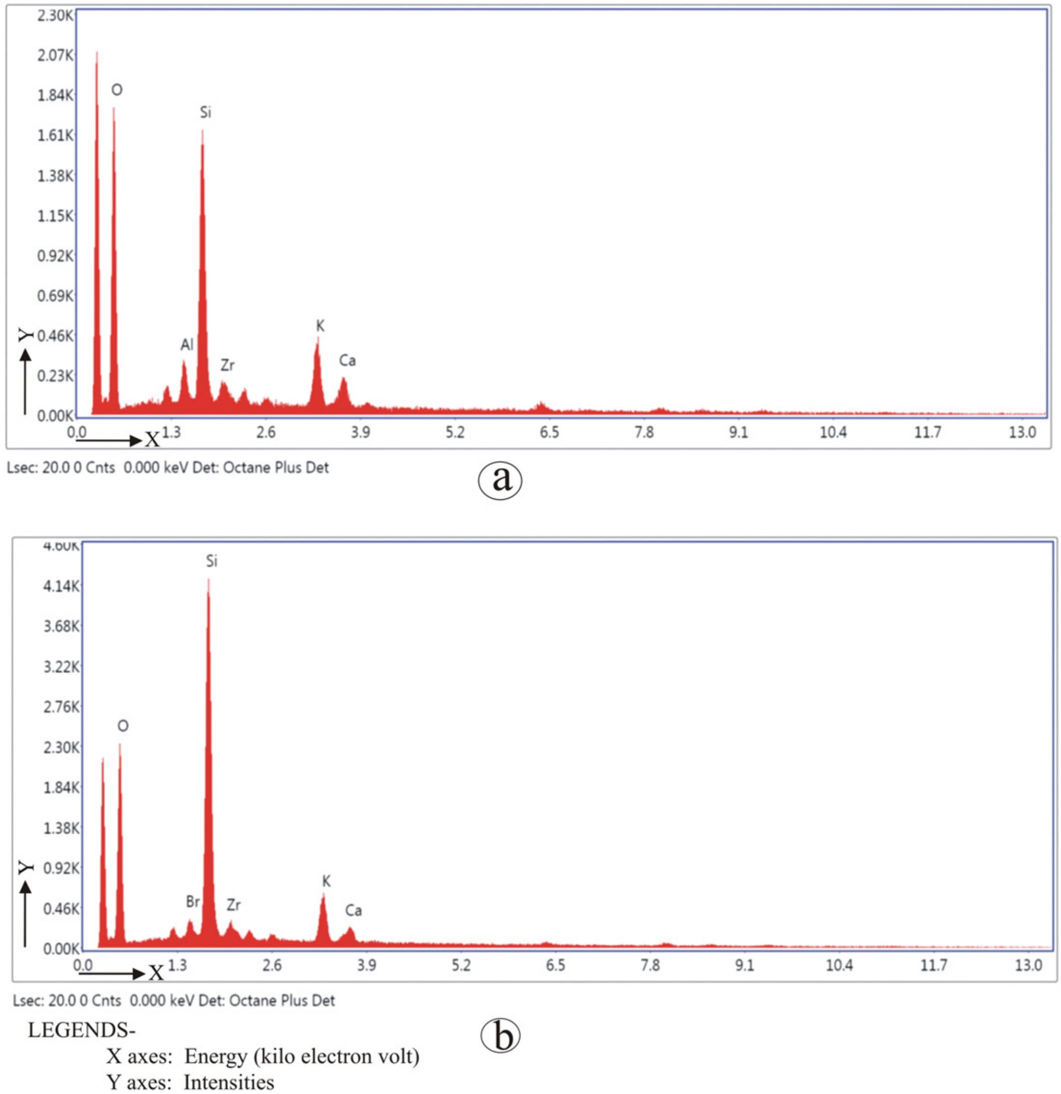

Fig. 8. The Field Emission Scanning Electron Microscope with Energy Dispersive Spectroscopy (FESEM-EDS) analysis micrographs in rhino dung collected from forest (a) and grassland (b) area in Kaziranga National Park.

some samples. *Rhododendron* does not grow in the area but does grow luxuriantly in the eastern Himalaya at least 500 km away from the study area. Its presence is interpreted as a strong indication of the annual flood activity in the region, since pollen of these taxa can be fluvially transported due to its entemophilous nature (Stephenson et al., 2007; Ranjitkar et al., 2014). So, the incorporation of this pollen into the dung might be through the rhino's ingestion of water as well as soil from the forest floor and swamp sediments, all of which are subject to frequent flooding. However, the pollen, especially of the upland taxa, may have been deposited on the rhino dung through the wind transportation from the higher altitude.

The dominance of grass pollen along with marshy and aquatic taxa is strongly indicative that these plants constituted an important component of the rhino's diet and were preferentially consumed. The marshy and aquatic taxa, chiefly Cyperaceae, *Polygonum*, *Nymphaea*, and *Eichhornia*, are indicative of the presence of perennial water-logged habitat in the park. This study confirms that the Poaceae forms the main part of the diet of the rhino, as indicated by the dominance of grass pollen in both the forested (27.7%) and grassland (31.1%) samples. The comparatively high abundance of marshy and aquatic taxa that are present in the perennially water logged condition in and around the area indicates their importance and that they are also essential parts of the rhino's habitat.

Apart from the pollen grains, the other palynomorphs, such as fungal spore, ferns, diatom, algal remains, Thecamoeba, and phytoliths were also observed and provide independent confirmation not only of the

Table 1
List of the elements value generated by FESEM-EDS analysis in rhino dung sample collected from forest area.

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
O K	64.58	79.13	516.57	9.15	1.0448	0.1961	1.0000	0.9732	0.2906
AlK	1.90	1.38	55.06	14.33	0.9308	0.0113	1.0146	1.0139	0.6310
SiK	18.67	13.03	629.76	4.91	0.9514	0.1326	1.0067	1.0206	0.7414
ZrL	3.28	0.70	44.65	20.79	0.7335	0.0215	1.0153	1.2112	0.8795
K K	7.65	3.84	187.93	6.62	0.8826	0.0638	1.0217	1.0487	0.9252
CaK	3.92	1.92	80.76	10.86	0.8988	0.0330	1.0174	1.0533	0.9200

Table 2
List of the elements value generated by FESEM-EDS analysis in rhino dung sample collected from grassland area.

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
O K	55.71	72.06	701.64	9.22	1.0560	0.1582	1.0000	0.9666	0.2690
BrL	1.39	0.36	40.46	21.24	0.7531	0.0099	1.0216	1.1791	0.9226
SiK	29.41	21.67	1744.46	3.94	0.9624	0.2182	1.0054	1.0150	0.7670
ZrL	3.95	0.90	83.40	11.90	0.7420	0.0238	1.0124	1.2049	0.8035
K K	6.92	3.66	278.55	5.16	0.8931	0.0562	1.0177	1.0440	0.8933
CaK	2.62	1.35	89.69	12.78	0.9096	0.0218	1.0170	1.0488	0.8993

rhinoceros' diet but also the presence of suitable habitat for the animal. Among the fungal remains in the rhino dung there is an abundance of the coprophilous fungi, *Saccobolus*, *Sporormiella*, and *Ascodesmis*. Their presence is significant as it strongly indicates a close host relationship provided by the rhino dung. These fungal spores are a strong indication of herbivorous animal dung (Lundqvist, 1972; van Geel et al., 1981, 1983, 2003; Bell, 1983; Hanlin, 1990). However, it must be noted that the *Cercophora* spore-type is a good dung indicator in relation to the presence of both woodland (Blackford and Innes, 2006) and grassland (Graf and Chmura, 2006) environments. Besides the coprophilous fungi the presence of other fungal remains, especially *Helminthosporium*, Microthyriaceae, and *Alternaria*, are common pathogens of herbaceous plants, particularly grasses. The regular presence of *Tetraploa*, in the assemblage reflects is general occurrence on the plants, usually on leaf bases and stems (Ellis, 1971). The abundance of *Glomus* with hyphae was observed in the assemblage and its occurrence is indicative of the endomycorrhizal fungi living in the plant roots of a variety of host plants (van Geel et al., 1989).

The abundance of fern, primarily *Polypodium*, *Dryopteris*, *Lycopodium*, and *Pteris*, in the palynomorphs assemblage indicates primary ingestion by the rhinoceros and ecologically is indicative of the warm and humid condition in the region. The abundance of fern spores was comparatively higher in the forested samples than the grassland samples. The consistent representations of algal remains, and Thecamoeba, chiefly *Botryococcus*, *Arcella*, and *Cyntropyxis* in the palynomorphs assemblage are strongly indicative of the presence of bodies of perennial fresh water in the region in response to the high annual precipitation. The presence of the diatoms *Synedra*, *Cymbella*, and *Cyclotella*, in the assemblage is also indicative of the fresh water-logged condition in response to the high rainfall in the region. The incorporation of this microbiota in the rhinoceros' dung would have resulted from the ingestion of water and soil in and around the vicinity of the water bodies.

The consistently high abundance of grass phytoliths in the assemblage was also observed and directly supports our pollen data that the grasses are the primary food for rhino. As expected grass phytoliths are comparatively higher in the grassland samples than the forested samples. The phytoliths represent localized signals in the palynomorphs assemblage (Piperno, 2006) which exactly support our data. There was a high recovery of C_4 grass phytoliths, in both the grassland (68.0%) and forest (58.6%) assemblages. The abundance of the C_4 grass phytoliths is a strong indication of the warm and humid climate in the region (Boyd, 2005; Barboni et al., 2007; Edwards and Still, 2008). In comparison, based on the FESEM-EDS elemental analysis, the proportion of grass phytoliths and Si content in grassland rhino dung samples (29.41%) was comparatively higher than the samples from the forested area (18.67%). Silica constitutes 2–5% dry matter in the leaves of grasses which is10–20 times higher than in the leaves of dicotyledonous plants (Russel, 1961). The silica contained in grass serves as a defense against both fungi and herbivores (Massey et al., 2006). The high value of grass phytoliths and silica in the rhino dung is useful as it can be used to differentiate the rhino dung from the dung of other herbivorous animals in the region.

Historically, based on the literature, the distribution of the habitat of the Greater one horned rhinoceros included western and northern India and Pakistan from 2500 to 1200 BC, but the species is now extinct in this region today due to human activity (Banerjee and Chakraborty, 1973). Based on phytoliths, it was proposed that the weak SW monsoon and high winter rainfall leading to cool climate during the mid-Holocene (3960 cal yrs BP) in western India resulted in an increased proportion of C_3 grass phytoliths compared to the C_4 grass phytoliths (Singh et al., 2007). Therefore, the vegetation and climate in relation to the weak monsoon at this time may be one of the primary reasons for the extinction of rhino in western India. The recorded palynodata of modern rhino dung can provide a critical baseline on the relationship between the distribution of the rhino and specific types of vegetation and it may be possible to infer the previous existence and distribution of suitable rhino habitat in the recent past through a study of sedimentary soil profiles and coprolite study, even when skeletal remains of the species are not available. The determination of the extent of former suitable natural habitat of the Greater one horned rhinoceros in the past may permit us to identify the underlying reasons as to whether climatic and resultant changes in vegetation or anthropogenic activity contributed to their extinction in the region.

6. Conclusions

The generated database of pollen recovered from modern rhino dung will be very useful to reconstruct the palaeovegetation and climate in relation to the rhinoceros dietary record. The representation of the arboreal taxa, though in low values, both in forested (16.2%) and grassland (7.7%) habitats and composed of a mixture of chiefly evergreen and deciduous taxa, *Mesua*, *Duabanga*, *Salmalia*, and *Dillenia* strongly signifies the presence of dense evergreen and deciduous forests in the national park, even though the arboreal taxa were not the primary food for rhinoceros. This palynodata can also provide a baseline that will permit the palaeoecological reconstruction and the former presence of suitable rhino habitat and preferred plants in its diet through pollen and phytoliths in sedimentary soil profiles. The generated palynodata also reflected that the rhino preferred different types of forest vegetation, along with grassland areas and water logged condition as part of the habitat they utilized. Lastly, these pollen and non-pollen palynomorphs data will be very helpful to trace the past distribution of the types of vegetation indicative of the preferred habitat of the rhino in India and adjacent areas in relationship to the present distribution. This approach can contribute to a better understanding its disappearance from former parts of its range and whether the cause of its extinction was the result of climatic change or anthropogenic activity.

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