Multiproxy studies on dung of endangered Sangai (*Rucervus eldii eldii*) and Hog deer (*Axis porcinus*) from Manipur, India: implications to paleoherbivory and paleoecology

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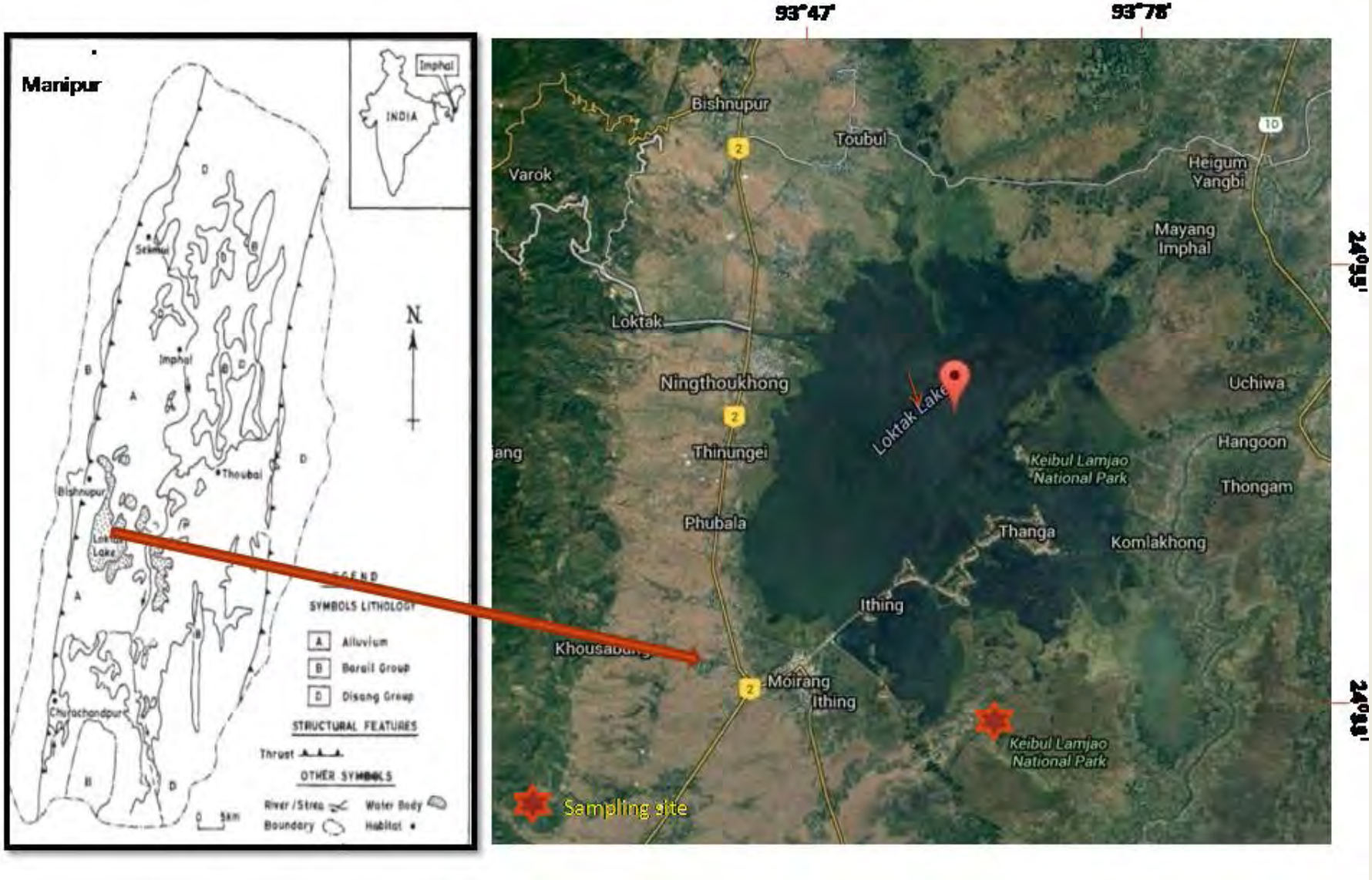
The Manipur Brow-antlered deer or Sangai (*Rucervus eldii eldii*) is the state animal of Manipur and leitmotif of the state's art, culture and folklore. It is considered to be 'Critically Endangered' under The IUCN Red List of Threatened Species (Gray et al., 2015) and is on the highest list of protection under the Schedule I of the Indian Wildlife (Protection) Act, 1972.

Its critically threatened status is the result of untreated human waste entering the 40 sq. km., national park, the deer's last refuge in India. This water includes liquid effluents, solid wastes and sewage from Manipur's capital city Imphal transported by polluted rivers that empty into the national park.

The deer was actually thought to have gone extinct until a remnant population was rediscovered in 1950 at the south-eastern fringe of Manipur's Loktak Lake. Besides, Sangai deer, a detailed assessment of the Red List status of *Axis porcinus* (common name Hog Deer) in 2008 convincingly demonstrated that the species had been undergoing a serious and overlooked global decline for decades.



Location map showing the study area (after Google earth)

Keibul Lamjao National Park


Keibul Lamjao National Park

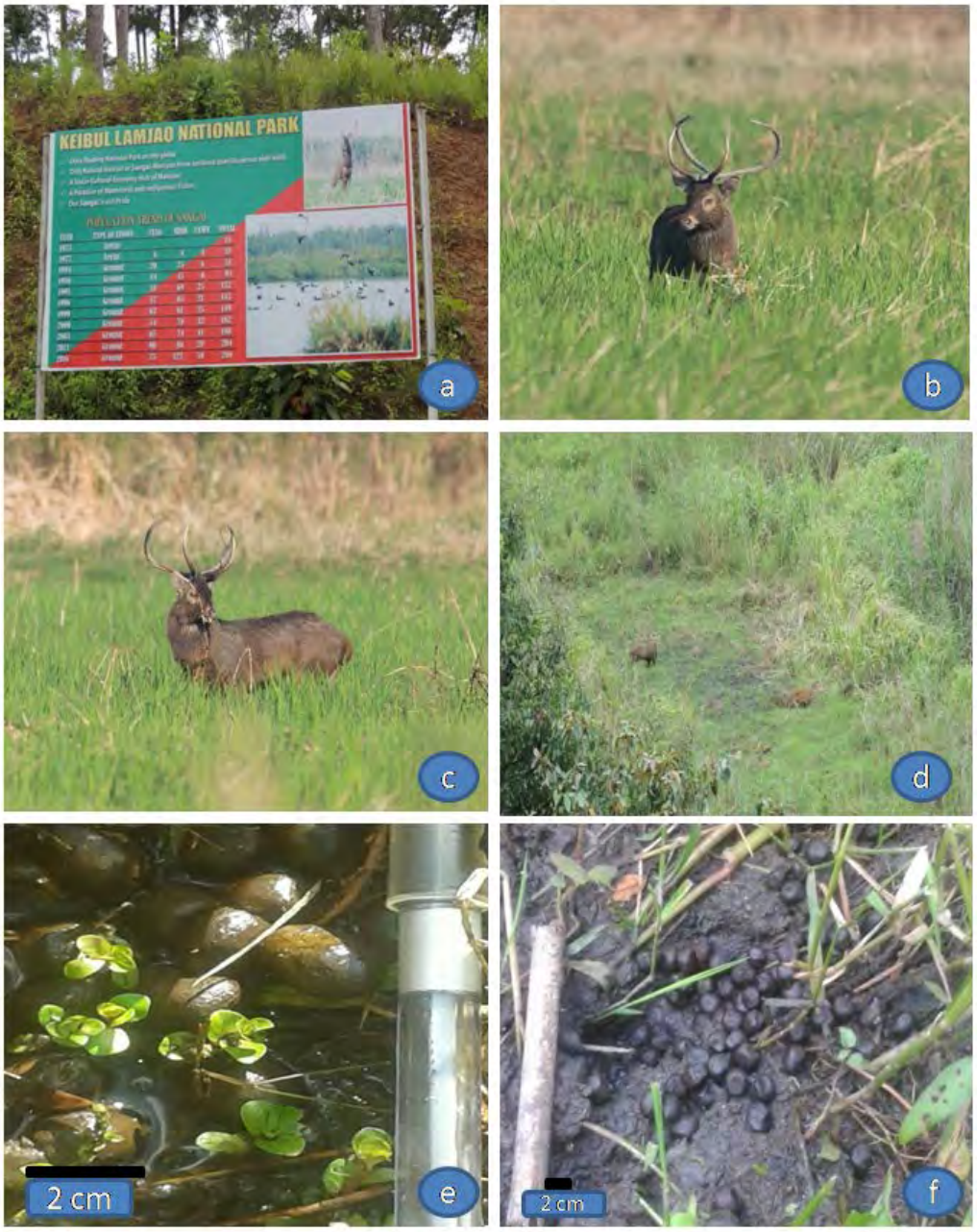

Loktak Lake (Ramsar site)

STUDY SITE, FLORA AND FAUNA

✓Located in northeast India, Keibul Lamjao National Park is a small area of around 40 sq. km., lying between latitude 24°27' to 24°31' N and longitude 93°53' to 93°55' E in the Bishnupur District of the state of Manipur in India (Fig. 1). It ranges in elevation between 700 to 900 m asl. The national park is characterized by many floating decomposed plant materials locally called phumdis (phumdis are a series of floating islands, made up of heterogeneous masses of vegetation, soil and organic matter, in different stages of decay), hillocks and elevated strips of land are also present and comprise an integral part of Loktak Lake (Ramsar site). It is therefore unique as the only floating national park of the world.

✓The park fauna is diverse and includes 81 species of resident and migratory birds, 25 species of reptiles and 22 species of mammals including Sangai and Hog deer (Trisal and Manihar, 2004).

✓The park was initially declared to a sanctuary for the deer in 1966, and was subsequently made a national park in 1977 in order to provide a natural refuge for the endangered Manipur Eld's deer or brow-antlered deer or Sangai also called the dancing deer. Vegetation in the park is primarily composed of tropical moist deciduous to semi–evergreen forests and has a rich amalgam of aquatic, wetland and terrestrial ecosystem.



Field Photographs

(a) Sign Board of Keibul Lamjao National Park,

(b) Sangai deer (*Rucervus eldii eldii*),

(c) Hog deer (*Axis porcinus*),

(d), view of lush grassland habitat utilized by herbivores in Keibul Lamjao National Park,

(e) dung samples of Sangai deer (*Rucervus eldii eldii*),

(f) dung samples of Hog deer (*Axis porcinus*) collected from Keibul Lamjao National Park.

INTRODUCTION

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).

Recovered palynomorphs sometimes indicate the pollen and spore release time, flowering season and season of ingestion of the consumed plant species (Ghosh et al., 2008).

Pollen and opal phytoliths are two of the most likely types of microfossils to be preserved in coprolites (Prasad, 2005). Many pollen taxa can be identified to specific families, genera or species.

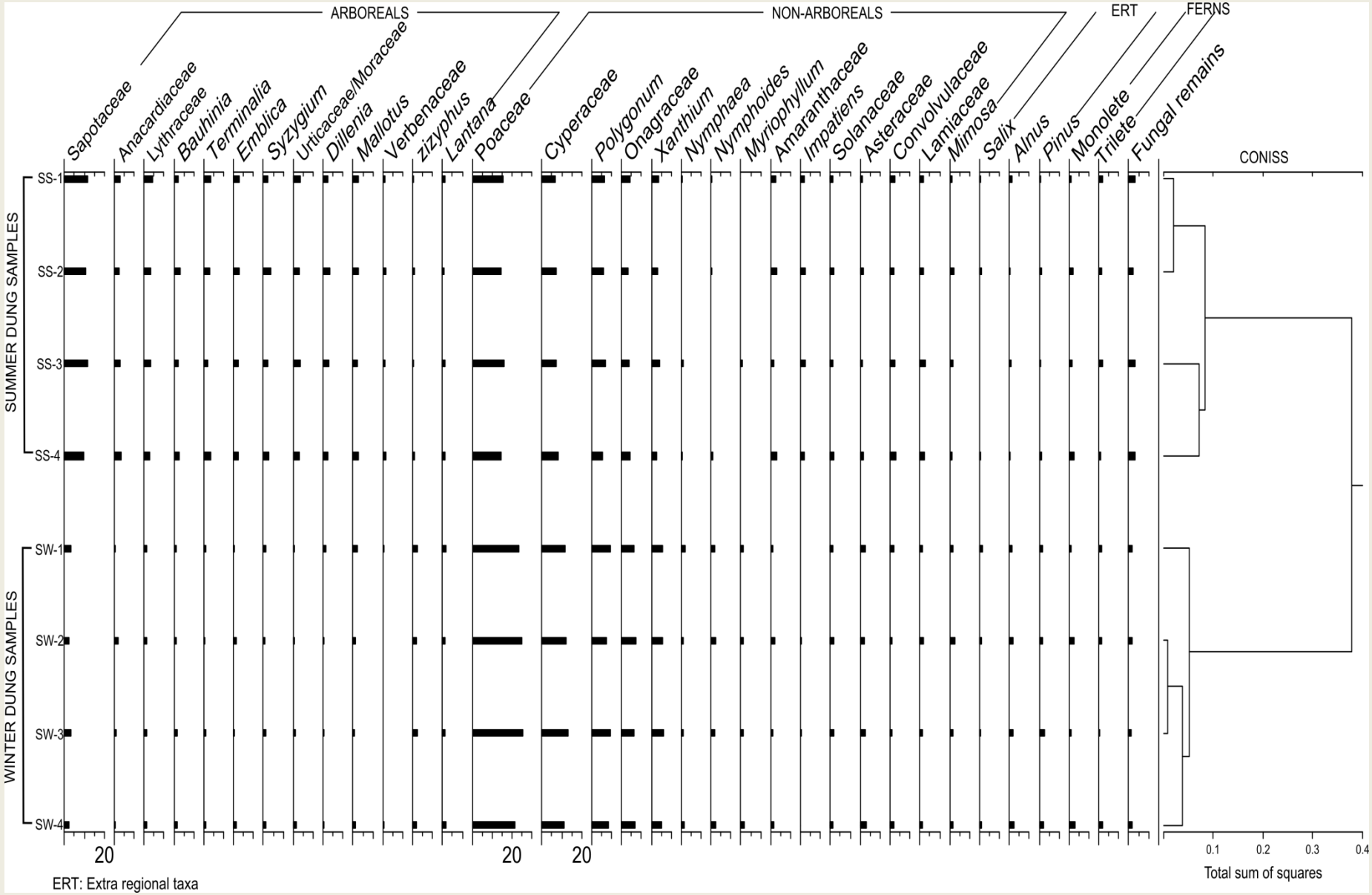
The outer wall of pollen grains and spores of ferns and fern allies are composed of sporopollenin, an organic substance highly resistant to decay (Faegri and Iversen, 1975). Opal phytoliths are highly durable silica bodies found in some plant tissue (Piperno, 1988).

Studies of coprolite palynology, especially in North America, have been used to interpret the prehistoric diet of both people and animals and to determine the local vegetation (Hall, 1972; Schoenwetter, 1974). Likewise, the modern pollen analogue of herbivores dung could therefore, also contribute in providing information pertaining to their food habit, vegetation composition and ecology.

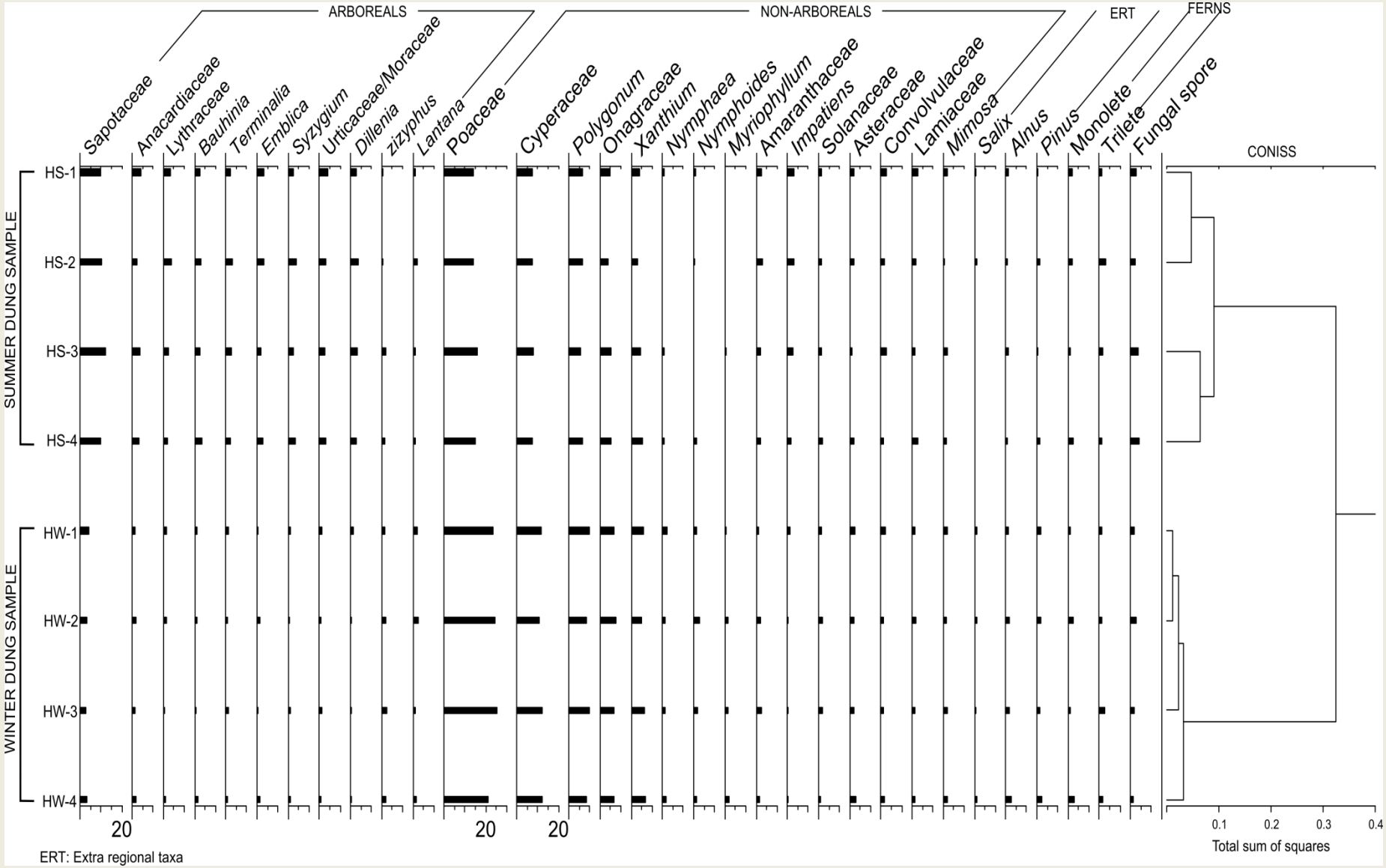
However, until recently, very limited research has been carried out in India on the pollen and non-pollen palynomorphs present on the dung of wild animals in relation to the local vegetation composition, and ecology in the region in conjunction with a dietary analysis of the different vertebrate species in the region.

One of the initial efforts has been to record pollen and non-pollen palynomorphs on the dung of the Greater One-Horned Rhino in Kaziranga National Park, India (Basumatary et al., 2017; Basumatary and McDonald, 2017).

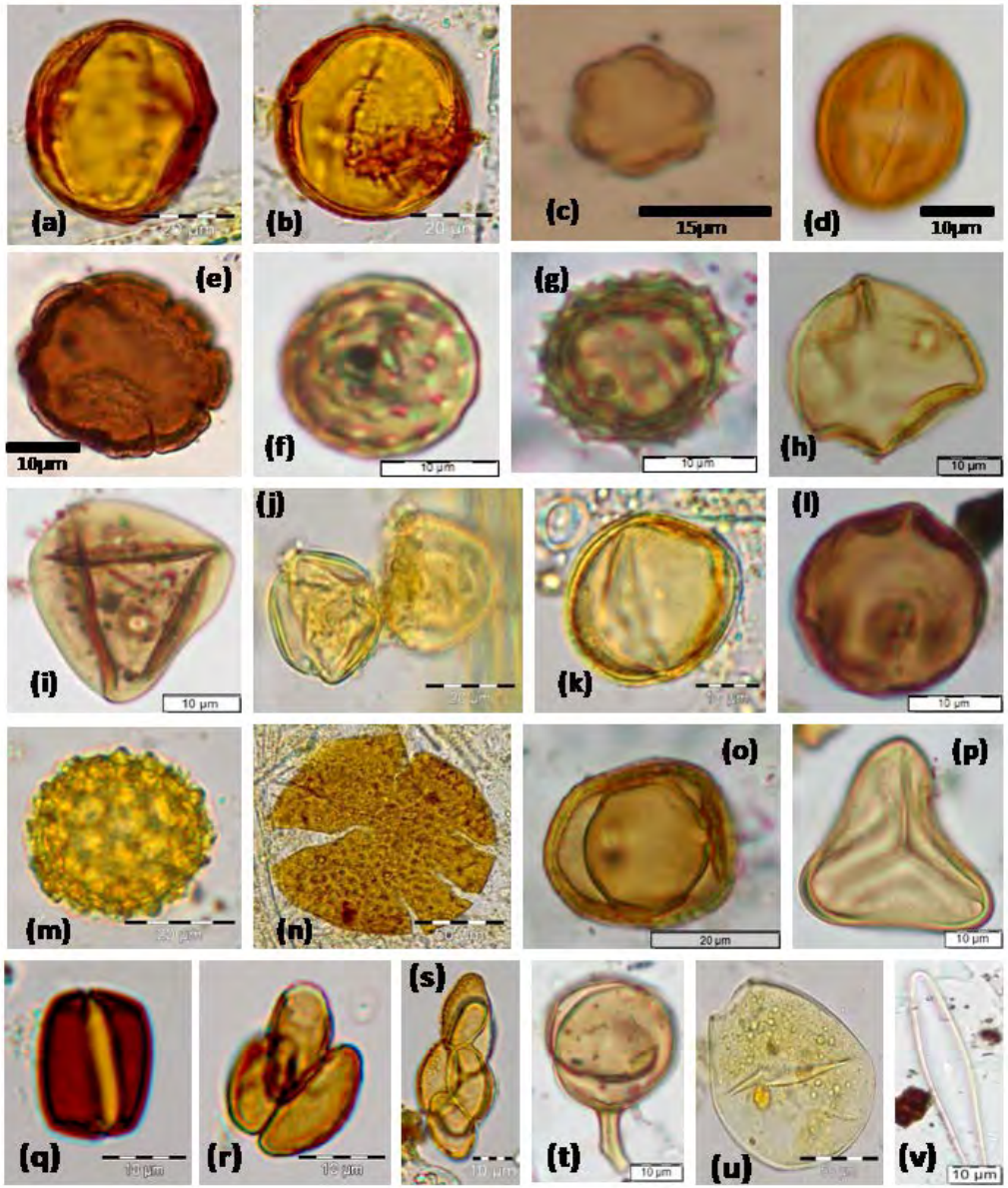
Therefore, the study of pollen and non-pollen palynomorphs from the Sangai and Hog deer dung has the potential to examine the relationship between the modern pollen and vegetation and the diet of these deer species and document differences in habitat utilization by these two cervids as well as seasonal differences in their respective diets.



Pollen spectra generated from dung of Sangai deer (*Rucervus eldii eldii*)



Pollen spectra generated from dung of Hog deer (*Axis porcinus*)



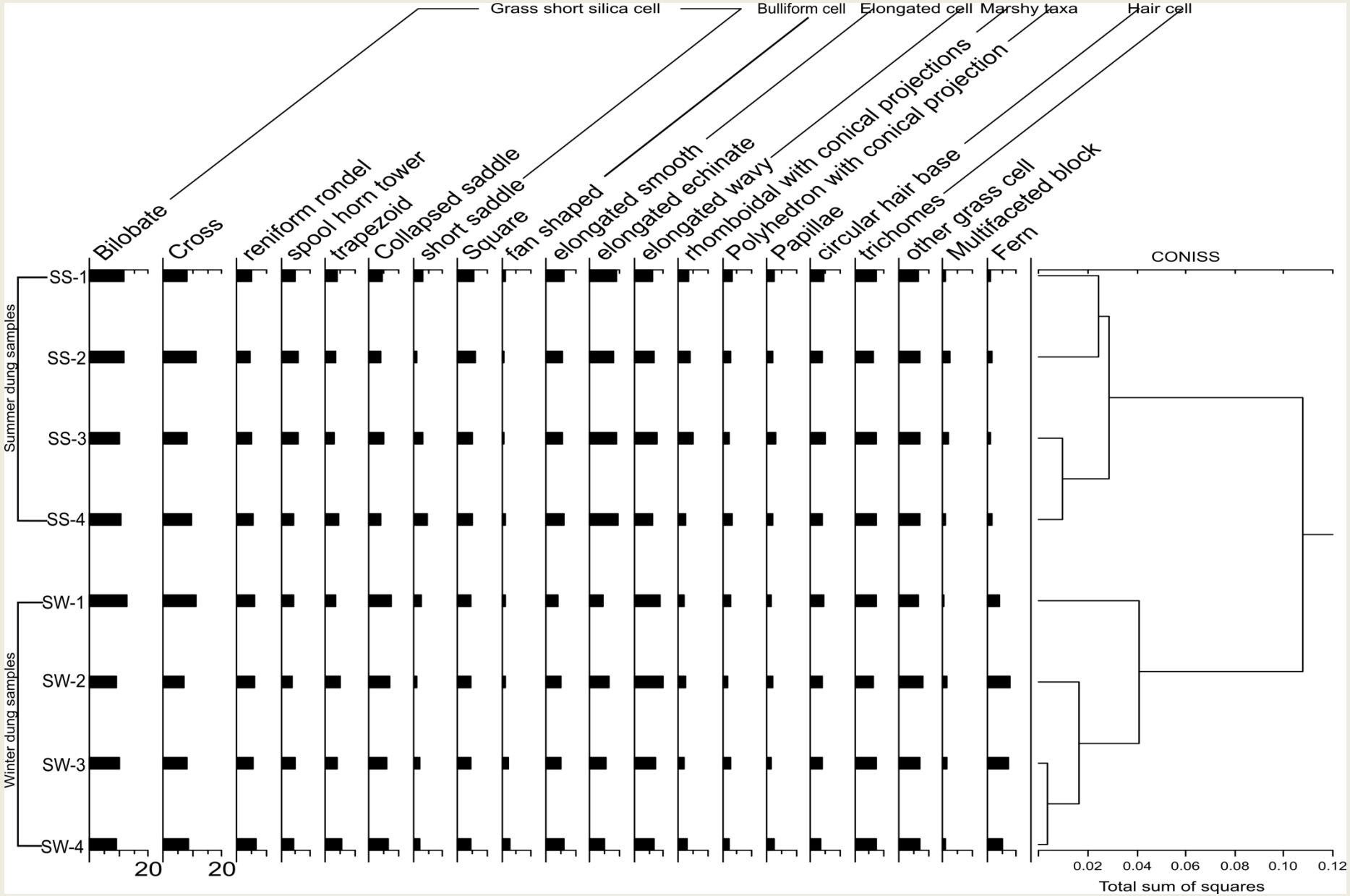
(a) & (b). Sapotaceae, (c). *Terminalia*,
(d). Anacardiaceae, (e) Convolvulaceae,
(f). Amaranthaceae, (g). Asteraceae,
(h) & (i). Poaceae, (j) & (k). Solanaceae,
(l). *Mallotus*, (m). *Polygonum*,
(n). *Salvinia*, (o). *Xanthium*,
(p). Trilete spore, (q). *Sporomiella*,
(r) & (s). Clumping of *Saccobolus*,
(t). *Glomus*, (u). *Nebela* (thecamoeba),
(v). *Pinnularia* (diatom).

Pollen and NPPs retrieved from dung samples of Sangai (*Rucervus eldii eldii*) and Hog deer (*Axis porcinus*)

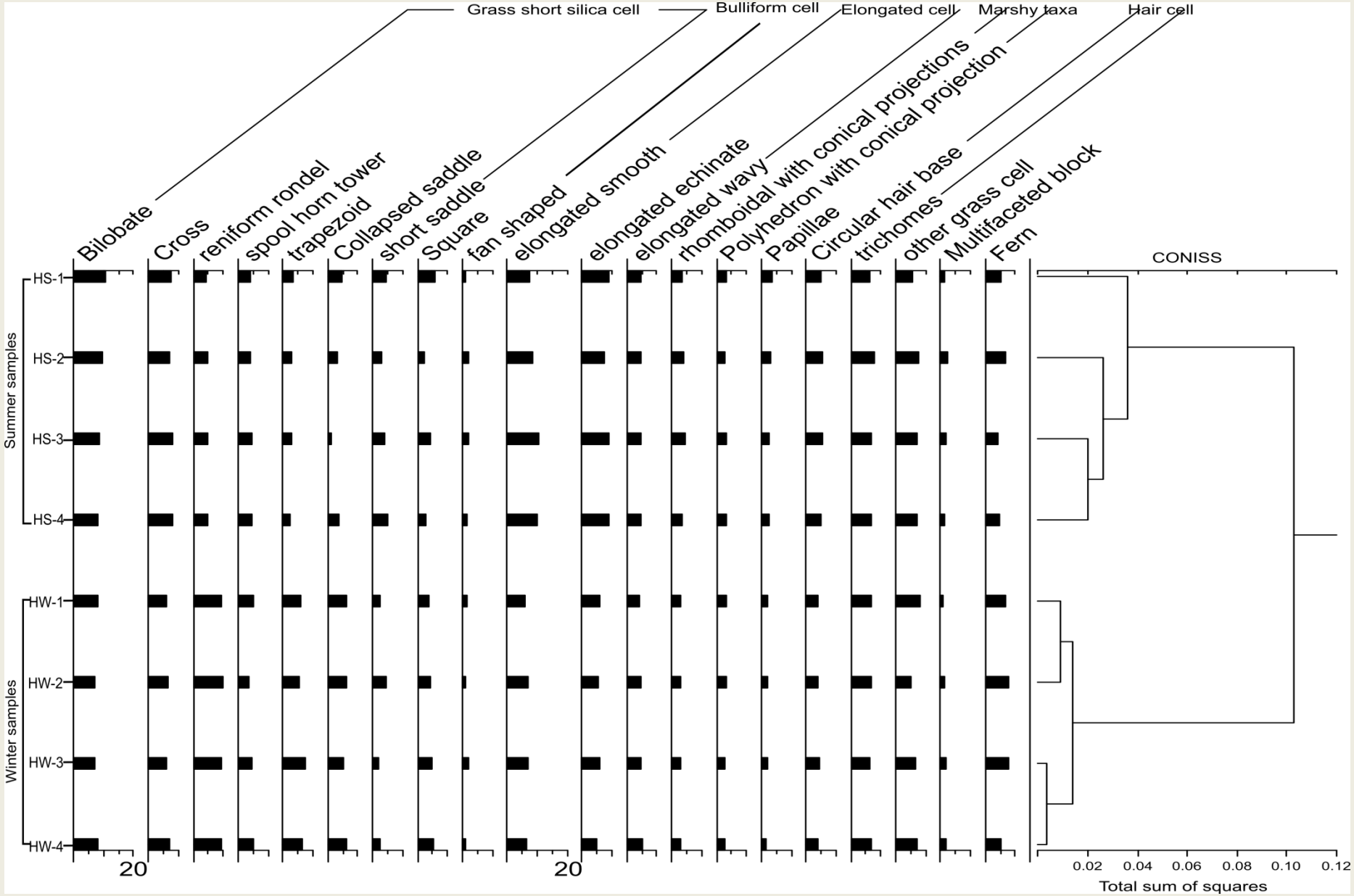
Chemical analyses

The samples for pollen analysis were processed employing the standard acetolysis method (Erdtman, 1953). The procured samples were treated with 10% aqueous KOH solution to deflocculate the pollen/spore from the sediments followed by 40% HF treatment to dissolve silica content. Thereafter, the conventional procedure of acetolysis was followed using acetolysis mixture (9:1 anhydrous acetic acid and conc. H2SO4). Finally the material was kept in 50% glycerine solution with a drop of phenol.

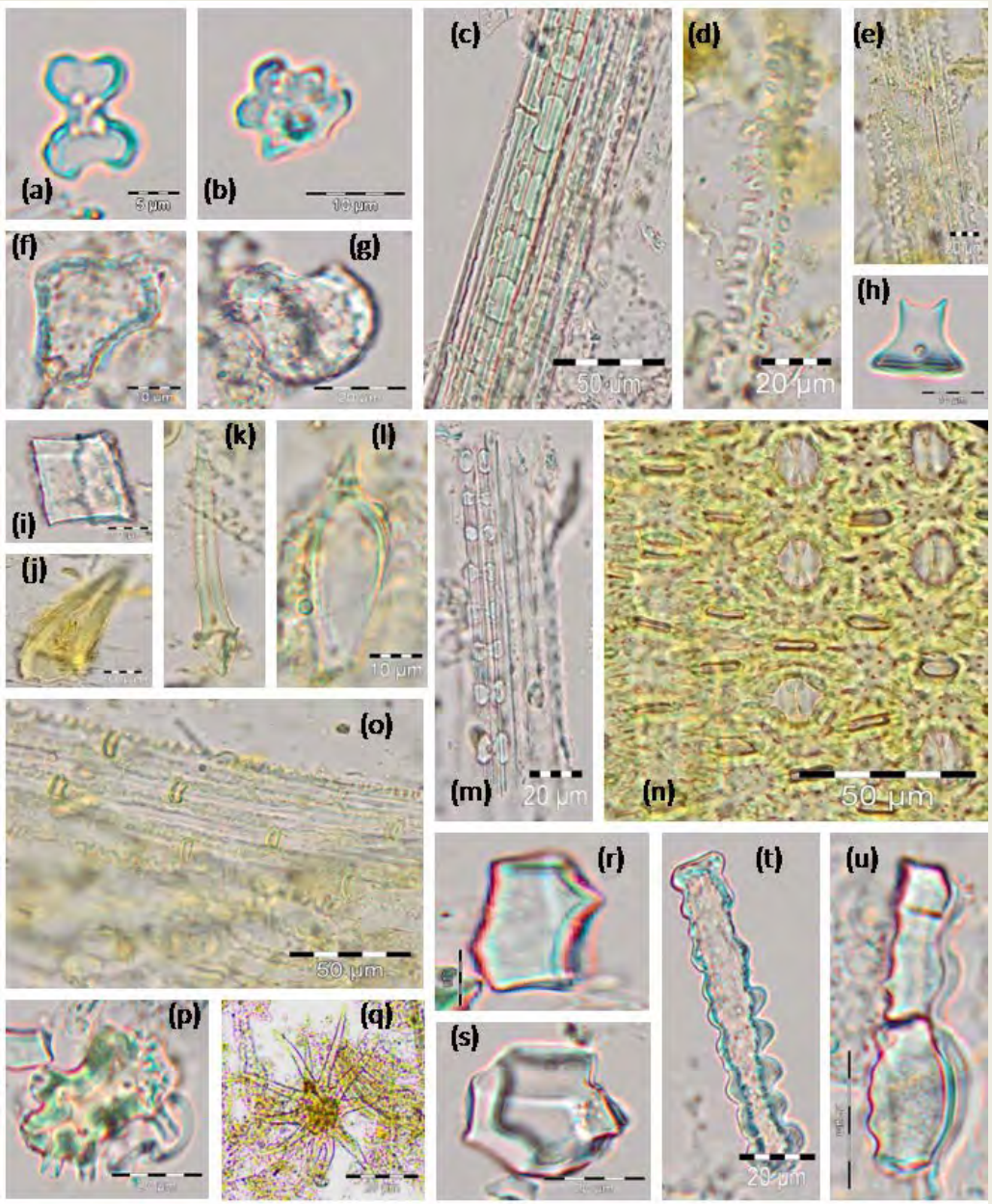
Phytoliths, being inorganic in nature resist decay and serve as a useful tool for palaeoecological and palaeodietary studies (del Puerto et al., 2006; Prasad et al., 2007; Bremond et al., 2008). Extraction of phytoliths was done following the standard technique of Piperno (2006) and Prasad et al. (2007) after slight modifications. A 20 g of dried dung sample was subjected to hydrochloric acid (HCL) followed by hydrogen peroxide (H_2O_2) to remove carbonates and organic content respectively. Extraction of phytoliths was carried out using CdI_2 and KI (specific gravity 2.3) and the slides were prepared using Canada balsam.



Phytolith spectra retrieved from dung of Sangai deer (*Rucervus eldii eldii*)

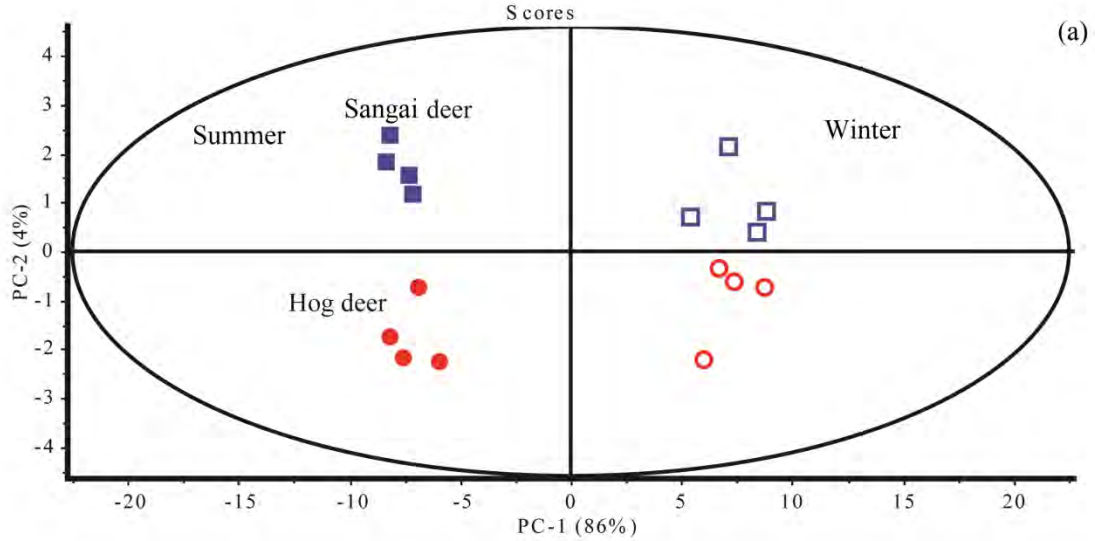


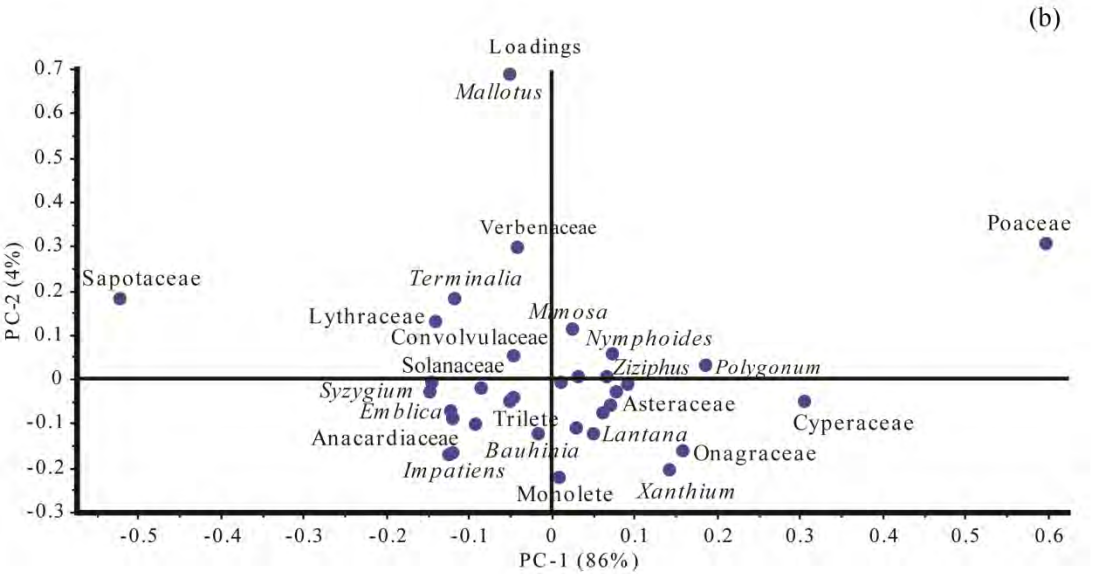
Phytolith spectra retrieved from dung of Hog deer (*Axis porcinus*)



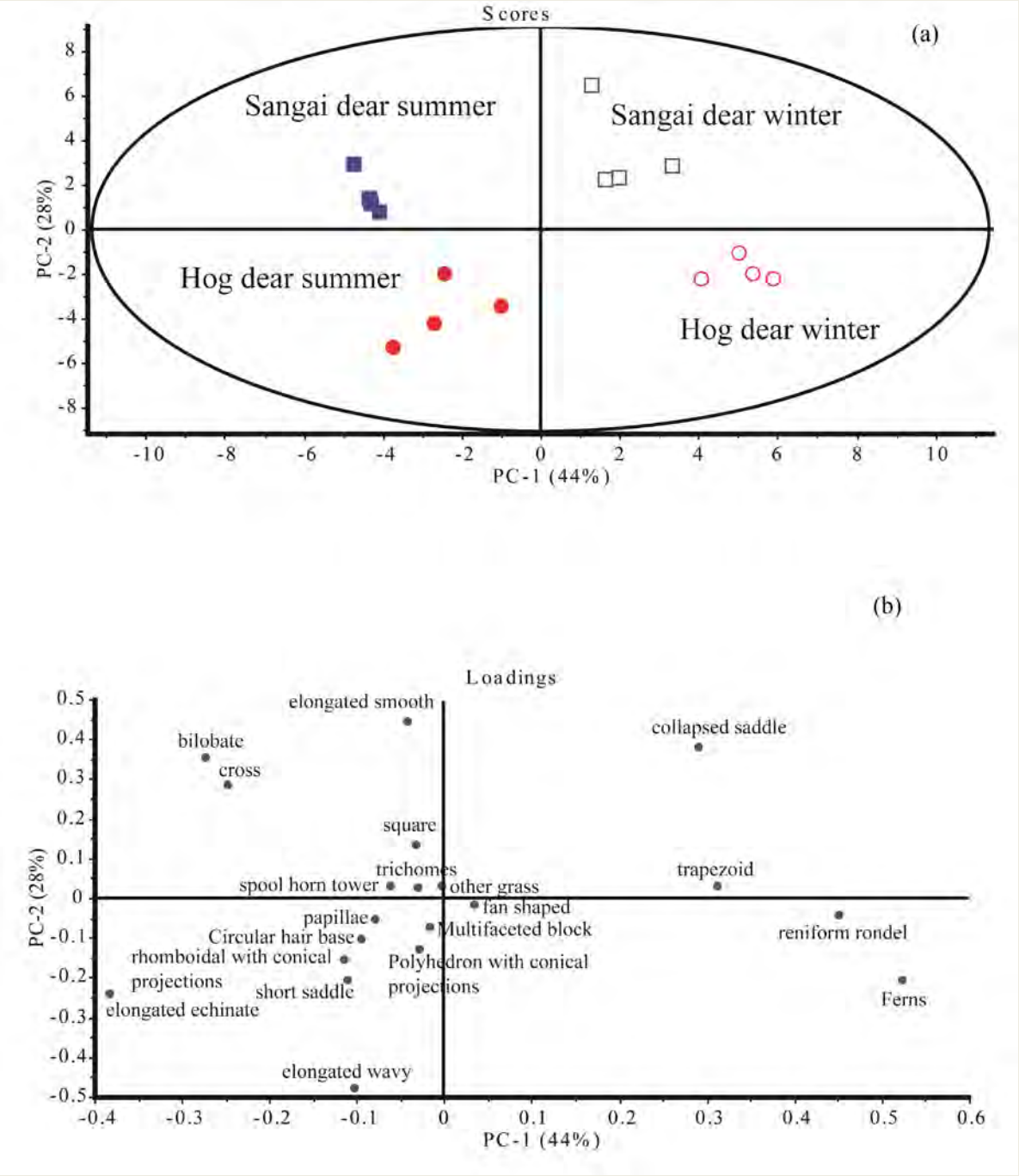
(a). bilobate, (b). cross, (c). an aggregate of bilobate and cross, (d). elongated grass cell, (e). an aggregate of elongated grass cell, (f) & (g). bulliform cell, (h). spool horn tower, (i). collapsed saddle, (j),(k) & (l). silicified trichomes, (m). an aggregate of bilobate cell, (n). silicified stomata group with interstomatal cells, (o). elongated grass silica cells with few reniform rondels (possibly *Zizania* sp.), (p). papillae, (q). silicified stellate hair, (r). polyhedron with conical projections, (s). rhomboidal with conical projections, (t) & (u). Fern phytolith

Phytolith morphs retrieved from dung samples of Sangai (*Rucervus eldii eldii*) and Hog deer (*Axis porcinus*)





(a) PCA score plot obtained from the combined PC analysis of different pollen frequencies retrieved from summer and winter dung samples of Sangai and Hog deer, (b) Corresponding loading plot

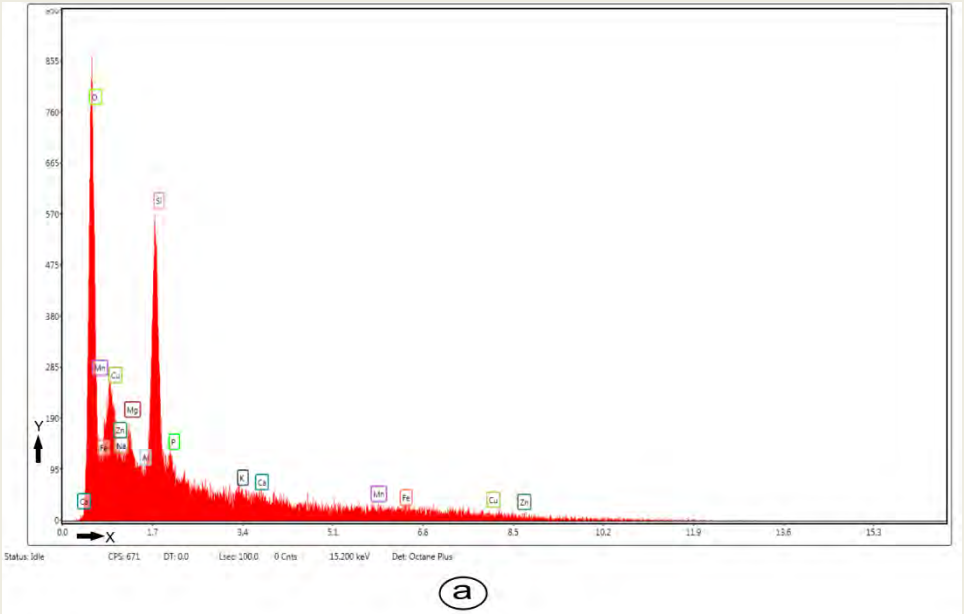


(a) PCA score plot obtained from the combined PC analysis of different phytolith morphotypes retrieved from summer and winter dung samples of Sangai (*Rucervus eldii eldii*) and Hog deer (*Axis porcinus*), (b) Corresponding loading plot.

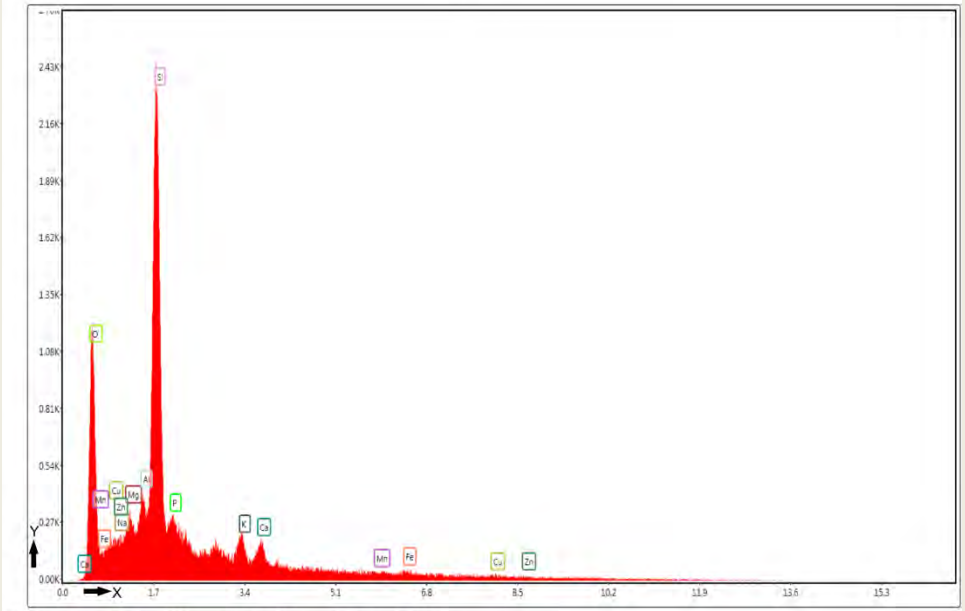
Inferences from PCA score plot

A clear cut seasonal clustering of these pollen taxa is present and mainly represented by two major pollen groups, arboreal and marshy/aquatic taxa. The major arboreal group like Sapotaceae, Lythraceae, *Terminalia*, *Syzygium*, *Emblica* and Anacardiaceae were recorded in the summer samples of both the deer species, thus coincides with the blooming season of these plant taxa. The winter samples were dominated by Poaceae and marshy and aquatic taxa like *Nymphoides, Polygonum*, Cyperaceae and Onagraceae. The dominance of Poaceae, Cyperaceae, Sapotaceae and *Mallotus* in the PCA loading plot shows that they are preferred by both deer species.

The PC score plot also indicates, seasonal clustering based on the appearance of the phytolith morphotypes of different plant species especially the Panicoideae grasses and marshy/ aquatics (indicative of warm and humid climate) which dominate in the summer dung samples. Whereas, Pooideae grasses, characterized by reniform rondel, trapezoid and fern morphotypes (prone to cool and high humid climate) were relatively more frequent in the winter dung samples.



(a)

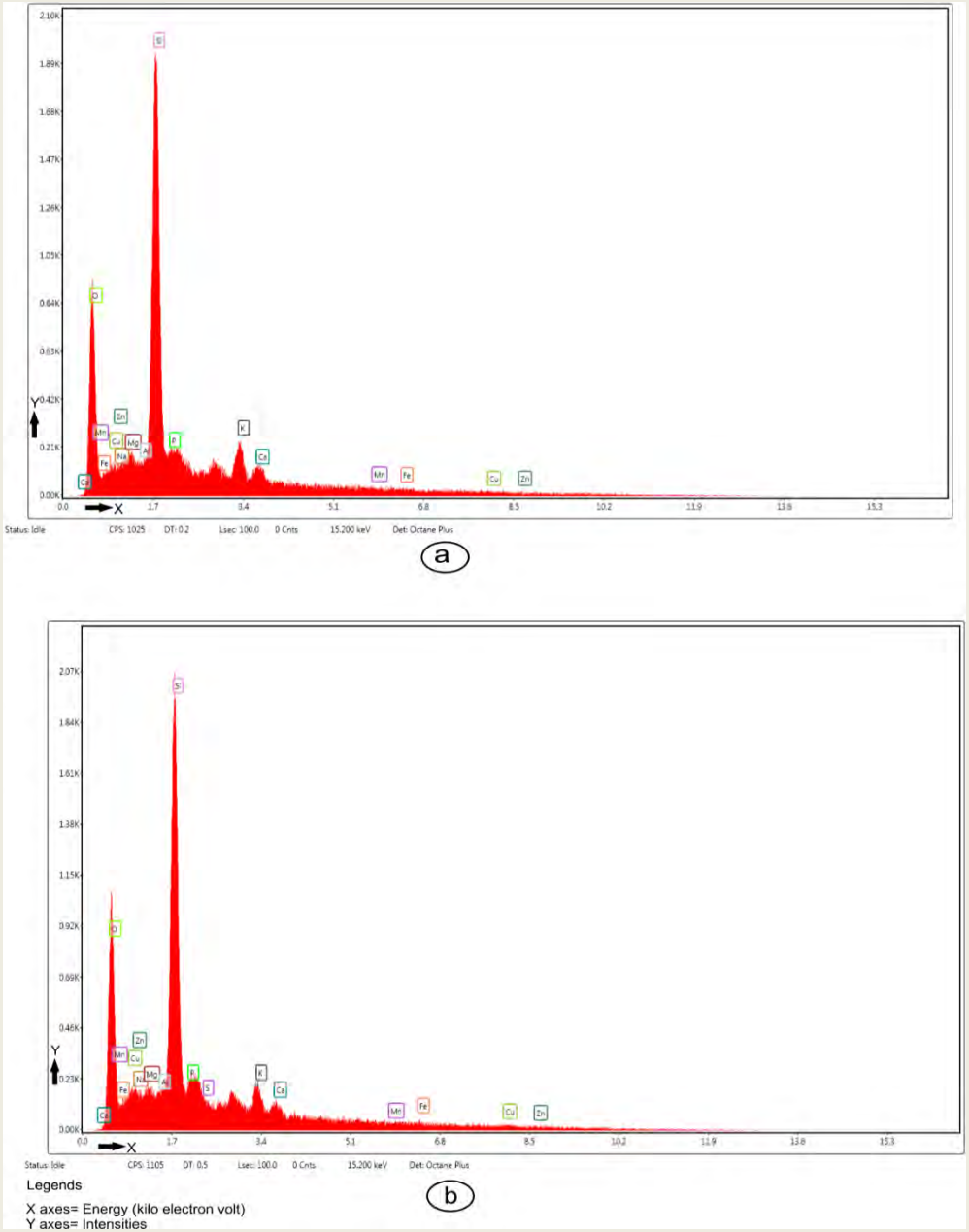


Legends

X axes= Energy (kilo electron volt)
Y axes= Intensities

(b)

The EDS spectra from summer and winter dung of Sangai deer (*Rucervus eldii eldii*)



The EDS spectra from summer and winter dung of Hog deer (*Axis porcinus*)

The FESEM-EDS elemental analysis of the two deer dung samples showed the dominance of Oxygen, followed by Silicon with relatively high frequencies in winter samples as compared to summer dung samples. This may be due to high diversity of grasses in winter season.

Major macro and micronutrients like phosphorus, aluminum, potassium, phosphorus, and magnesium were also noted in the dung samples for both seasons, but with minimal differences. However, potassium and phosphorus are always present at comparatively higher values in the Hog deer, which is significant and needs further examination.

MAJOR CONCLUSIONS

- **The generated pollen and phytolith dataset reveals that while grass is the primary food for these two endangered deer species, high plant diversity in their diet is necessary for their survival as reflected in both summer and winter dung samples.**

- **The recovery of arboreal taxa especially Sapotaceae along with marshy and aquatic taxa (*Polygonum*, *Impatiens,* Cyperaceae and *Nymphaea*) was very significant and indicative of the tropical mixed deciduous forest along with the perennial water logged condition in the region.**

- **The elemental analysis provides a better understanding of the nutritional value of the various plants in their diet in relation to the vegetation composition, climate and the availability of the preferred food plants.**

- **This data could help in the differentiation and characterization of the herbivorous dung in relation to the vegetation composition and preferential diet in the region.**

- **This generated data could also be a counterpart of the study dealing with modern pollen and vegetation relationship, because of hindrance in the systematic procurement of surface soil samples in the region due to seasonal flooding of the region.**

- **The generated data could also be a strong baseline to observe the palaeoherbivory and palaeoecological analysis in relation to the coprolite and palaeontological observation in the region.**

- **The pollen diversity is higher in the Hog deer in comparison to the Sangai deer and the difference might be due to the differential feeding habits. The Hog deer might obtain its food from the different feeding level ranging from ground to 3-4 feet in height, owing to its smaller body size and more frequent jumps. Thus, rivers and open grasslands provided the best grazing and sheltering ground for Hog deer (Bhowmik et al., 1999). While the comparatively larger size and body mass of Sangai deer could reflect the relatively lower plant diversity in its dung samples.**

- **The diatoms and phytoliths diversity were also comparatively higher in the Hog deer, which shows that this species had a wider feeding range within the study area.**

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