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Multiproxy analysis on Indian wild ass (*Equus hemionus khur*) dung from Little Rann of Western India and its implications for the palaeoecology and archaeology of arid regions

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ABSTRACT

The dung of the Indian wild ass was analyzed using biotic and abiotic proxies to determine its dietary habits in relation to the plant diversity and ecology in the arid region of western India. The presence of both micro and macrobotanical remains of Poaceae, Chenopodiaceae, and Fabaceae indicates they are the primary food plants of the wild ass. The continuous recovery of arboreal pollen taxa chiefly, *Prosopis, Acacia,* and *Ephedra* is indicative of dry thorny forest under semi-arid to arid conditions which display the existing vegetation and climate in the region. The recovery of marshy pollen taxa like Cyperaceae and Onagraceae along with *Arcella* indicates utilization of water-logged environments in the habitat. Spores of coprophilous fungi, *Sporormiella, Sordaria,* and *Podospora* are also present in the dung samples. The low value of stellate trichomes in winter dung samples reflects the seasonal migration of wild ass. Average $\delta^{13}C$ values ranging between –15.8‰ and–26.3‰ are indicative of a mixed diet of both C_3 and C_4 plants. The generated multiproxy data from dung samples can provide a reliable counterpart to modern data for the interpretation of the palaeoecology in relation to the palaeoherbivory and palaeodietary analysis in the region. This study also provides a basis to distinguish between wild and domesticated herbivores by analyzing coprolites and cultural sediments in archaeological sites.

Keywords: Indian wild ass, Pollen, Dung midden, Arid region, Archaeology, Western India.

1. Introduction

The Indian wild ass (*Equus hemionus khur*), an endangered subspecies of the Asian wild ass (Feh et al., 2002; Shah and Qureshi, 2007; Kaczensky, et al., 2016; Bennett et al., 2017) is today restricted to small areas in western India. Recent studies of the dung of endangered megaherbivores in tropical, subtropical and temperate regions of India to analyze their dietary patterns relative to the existing vegetation have been conducted as part of the modern analogue, species conservation, and restoration strategy (Basumatary and McDonald, 2017, Tripathi et al., 2019; Basumatary et al., 2019, 2020, 2021). Building on these previous studies of modern herbivore dung as well as on coprolites of extinct species (Bryant and Larson, 1968; Bryant, 1974; Riskind, 1970; Ghosh et al., 2008; Wood et al., 2008, 2021; Gill, et al., 2009, 2013; Velazquez and Burry, 2012; Marinova et al., 2013; Gravendeel et al., 2014; Rawlence et al., 2016; Birks et al., 2018; Harrault, et al., 2019) we have examined the dung of the Indian wild ass to better understand the species dietary plant preferences and ecology.

Likewise, the examination of botanical remains preserved in modern herbivore dung can also be applied to dung preserved in archaeological sites and can be a source of data for the interpretation of the palaeoecology, palaeovegetation, and climate in relation to the development of human cultures and their impact on the local environment and fauna (Akeret et al., 1999; Baeten et al., 2018, Smith et al., 2019, Rotunnu et al., 2019, Dunseth, et al., 2019; Fuks and Dunseth, 2021).

Previous palynological work on soils in northwestern India provides a basis to understand the modern pollen rain of the region and aid in reconstructing the palaeovegetation and climate (Singh et al., 1973). However, to date, no palynological work has been attempted on surface soil

of Kachchh and its neighboring areas for developing a similar modern pollen analogue for palaeoecological interpretation of this region. The systematic study of modern pollen rain from surface samples of arid and semi-arid region is difficult due to the scarcity of depositional environments like forests, swamps, lakes, and wetlands that favor pollen preservation (Horowitz, 1992; Gil-Rann et al., 2006). Dung can serve as a complementary data source and counterpart to more traditional sources of pollen in the arid regions (Scott and Bousman, 1990; Scott and Cooremans, 1992; Scott and Cooremans, 1992; Scott, 1996; Scott and Vogel, 2000; Carrión, 2002; Pearson and Betancout, 2002; Scott et al., 2004; Carrión, et al., 2006; Marinova et al., 2013; Basumatary et al., 2017, 2019). Dung as a substrate that preserves pollen in arid regions can also be sampled at different times of the year and thus can provide a basis to determine seasonal changes in plant diversity and phenology especially flowering and fruiting period of different plant species in this arid region. Such studies can augment scattered palaeoclimatic and palaeocological work that has been carried out in the arid-semiarid environment of Gujarat (Laskar et al, 2010; Pokharia et al., 2011, 2017, 2020; Farooqui et al., 2013; Prasad et al., 2014) in relation to the Indus and Harappan civilization (4600–3700 yr BP).

Stable carbon isotope analysis provides another approach to determine the preferences for C_3 and C_4 plants in an herbivore's diet in relation to the vegetation composition and climate in the region (Agarwal et al., 2012; Basu et al., 2015). Palynofacies analysis also aids in understanding their seasonal food habits. The palynofacies analysis of dung samples is subject to the dietary habits, the organic matter that the wild ass consumes (Hoaen, 2000; Medanic and Silva, 2010). It is observed that the seasonal vegetation is imparted as one of the feeding diets for the wild ass and hence the accumulation of dung primarily comprises the organic matter or vegetal remains that are eaten during the different seasons. Thus, the palynofacies in this way

helps to ascertain the seasonal organic matter in dung sample (Hoaen, 2000). In Mongolia the diet of the Asian wild ass is highly seasonal, and the species is primarily a grazer in the summer but a mixed feeder in winter (Sturm et al., 2017). Data on seasonal changes in elemental composition provides an indication of potential seasonal dietary stress resulting from changes in the availability of certain elements during part of the year (Levick et al., 2008; Waldram et al., 2008; van der Waal et al., 2008, 2011; Ripple et al., 2015). Previously, no research has been conducted on the organic and inorganic contents of the dung of the Indian wild ass in relation to the vegetation composition and climate of western India. The main aim of this study is to conduct a multiproxy analysis of Indian wild ass dung in order to better understand the ecology of the species and provide a baseline to determine the palaeoherbivory, palaeoecology, and archaeology of this arid region.

2. Geographical distribution of hemiones

Grubb (1993) recognized three species of hemiones, *Equus hemionus*, *E. kiang*, and *E. onager*. Only two species of hemiones, *Equus hemionus* and *Equus kiang* (Tibetan kiangs) were considered to represent the so-called Asiatic wild asses by Jónsson et al., (2014) and the onager was included as a subspecies of *E. hemionus*. Historically hemiones had a wide geographic range that included Mongolia, Central Asia, Anatolia, and Russia, but their current range is restricted to the border of Mongolia and China..

While the range of the *Equus hemionus* was greater in the past based on remains recovered from Middle and Late Pleistocene deposits of the Purna Basin in Maharashtra (Thakuri et al., 2018), today its range is restricted to the protected area in Little Rann of Gujarat, India as the population in Baluchistan became extinct over the past 40 years (Daniel, 1991). The

spatial distribution of the Indian wild ass on the landscape is largely influenced by the distribution of food patches and water in this ecosystem.

3. Study area, vegetation, and wildlife

The present study was conducted in the Little Rann of Kachchh (LRK) and its surrounding in the Gujarat State of India (Fig. 1). The Little Rann is characterized by vast desiccated bare mudflats consisting of dark silt encrusted with salts. The LRK is dotted with around 74 elevated plateaus or islands. Due to the distinctiveness of the area and the existence of the endemic and endangered Indian wild ass the LRK was established as "Wild Ass Wildlife Sanctuary" in 1973 (Pasha et al., 2015). It is the largest wildlife sanctuary in India. The Little Rann of Kachchh is a seasonally wet-saline biome, with plants including grasses growing in a saline environment. The vegetation of the study areas includes dry thorny scrub forest composed of *Prosopis juliflora*, *Carissa spinosa*, *Ziziphus nummularia*, *Salvadora oleoides, Salvadora persica, Grewia, Lantana camera*, and *Ephedra*. The main ground cover vegetation in the region includes many species of Poaceae, Cyperaceae, Onagracaeae, Chenopodiaceae, Malvaceae, and Amaranthaceae (Ishnava et al., 2011). The flora includes 372 species of flowering plants (Pandey, 2008).

Among wild herbivores, *Equus hemionus khur* is commonly seen in the region (Fig. 2a). Other herbivores including, *Boselaphus tragocamelus* (Blue buck), *Gazella bennettii* (Indian gazelle), and *Rusa unicolor* (Sambar) are also commonly found in association with the Indian wild ass. The fauna also includes 307 species of birds (Pandey, 2008).

4. Climate and soil

The climate of the region is influenced by south-west monsoons. Annual rainfall is 125 - 400 mm with most of it occurring from July to September (Sinha and Goyal, 2006). During summer it is hot with a maximum temperature reaching 48°C, while it is cold and dry in winter with the minimum temperature as low as 1°C. The soil types of the study are primarily blackish sand with low amounts of organic matter. Agricultural fields and wasteland extend up to the borders of the LRK.

5. Material and methods

5.1. Field Survey and sampling

During the winter (December-January) season of 2016-2017, AKP (first author) and team collected fifteen modern (fresh, 5-10 days old) dung samples (KW1-KW15). Dung selection was based on size and shapes so were considered to be from only adult population of wild ass (Fig. 2b). Similarly, in 2018-2019, GC (co-author) and team collected fifteen modern dung samples of the wild ass (KS1-KS15) during the summer (May-July) based on their size and shape. Each sample, consisting of approximately 200g, was collected from in and around the Little Rann of Kachchh (Lat. 23°22'58.05"N – 23°35'29"N and Long. 70°52'26"E – 71°5'26.99"E). The outer surface of the collected dung samples was removed and the inner portion of the dung was packed separately in polythene bags to prevent contamination by pollen dispersed by wind and other possible contaminates before laboratory processing. Finally, the collected samples were mixed finely and taken to process for the different proxy analysis as per the sample's requirement.

5.2. Laboratory work

5.3. Macrobotanical analysis

For the macrobotanical analysis, 50 g of each of the 30 (15 per season) dung samples were gently boiled in 200 ml 5% KOH solution. After boiling, material was sieved through a 150 µm mesh prior to selection for study. The material was washed 2 to 4 times with distilled water and observed under stereo microscope (Leica Z6APO) and photographed with a Leica DFC295 camera (Fig. 3). The total of macroremains were counted and relative proportion (percentage) of each taxa was calculated. Identifications were made using published literature (Martin and Barkely, 1961; Musil, 1963) and reference specimens in the herbarium of the Birbal Sahni Institute of Palaeosciences (BSIP), Lucknow, India.

5.4. *Microbotanical analysis*

5.5. *Pollen and non-pollen palynomorphs*

Thirty dung samples were processed for pollen and non-pollen palynomorphs (NPPs) using the standard acetolysis method (Erdtman, 1953). Samples were deflocculated using 10% solution of aqueous potassium hydroxide (KOH), treated with 40% hydrofluoric acid (HF) to dissolve silica, and acetolysis (9:1 anhydrous acetic anhydrite to concentrated sulfuric acid) for the removal of cellulose. The samples were washed twice with glacial acetic acid (GAA) and washed 3 to 4 times with distilled water. The samples were transferred to a 50% glycerol solution with a few drops of phenol to protect against microbial decomposition. 151 to 178 pollen and non-pollen palynomorphs (fungal spores, *Thecamoeba*, and algae) were counted from each sample to produce the palynomorph spectra. Results were plotted using TILIA software. The recovered pollen taxa were categorized as arboreal taxa (trees and shrubs), non-arboreal taxa (marshy and terrestrial herbs), fungal spores, *Thecamoeba*, and *Botryococcus*. For the identification of pollen grains, we consulted the reference slides at the Birbal Sahni Institute of

Palaeosciences herbarium as well as published literature (Gupta and Sharma, 1986; Nayar, 1990; Farooqui et al., 2013) (Fig. 4).

5.6. *Phytolith analysis*

For phytolith analysis 30 samples from the summer and winter season were analyzed using the standard technique (Piperno, 2006). A 10 g sample of dried dung was treated with hydrochloric acid (HCl) to remove carbonates followed by removal of organic content by hydrogen peroxide (H_2O_2). Extraction of phytoliths was performed using a mixture of CdI_2 and KI (specific gravity2.3) and placed on the slides using Canada Balsam. A total of 300–350 phytoliths were counted from each sample. Observation and microphotographs were done using an Olympus BX-61 microscope with DP-25 digital camera under 40x magnification (Supplementary Fig. 1). Phytolith morphotypes were grouped according to the standard classification (Madella et al., 2005; Twiss et al., 1969; Mulholland and Rapp, 1992).

5.7. *Palynofacies analysis*

For palynofacies analysis, ~5 g of each sample was treated with 10% HCl to remove the carbonates from the dung samples. Repeated washing 3-4 times using distilled water was done to neutralize the sample and subsequently the residue was treated with 40% HF solution to dissolve the silicates. The samples were again thoroughly washed, sieved (20 µm) and then the slides were prepared using polyvinyl alcohol and mounted using Canada Balsam (Tyson, 1995). No oxidizing agent was used during the processing of palynofacies analysis. The slides were then scanned under Olympus BH-2 microscope and photographs taken with DP-25 camera (Supplementary Fig. 1). The maceration process and literature consulted for the palynofacies

analysis follows Traverse, 1994; Pocknall and Beggs, 1990; Batten, 1996; Roncaglia, 2004, and Sebag et al., 2006.

5.8. *PCA analysis*

To obtain the vegetation and dietary differentiation in the dung samples procured from the Little Rann, Principal Component Analysis (PCA) was applied to the quantified pollen and phytolith frequencies analysis using CANOCO v.5 (Fig. 5) and the significance of the data was evaluated at p-value $\leq .05$ (Smilauer and Leps, 2014).

5.9. *FESEM-EDS analysis*

A Field Emission Scanning Electron Microscope (FESEM) with Energy Dispersive Spectroscopy (EDS) using FESEM (JEOL, JSM-7610F) equipped with EDS (EDAX, USA instrument) operated at 25 keV was used to determine the elemental composition of both summer and winter samples of the Indian wild ass dung (Fig. 6; Supplementary Table 1 and 2).

5.10. *Stable carbon isotope analysis*

For carbon isotope analysis ($\delta^{13}C_{org}$), ~1g of the dung sample was decarbonized using HCl (5%) for 2 hours in a water bath at 50°C and the procedure was repeated three times. Subsequently the samples were centrifuged, rinsed repeatedly with deionized water until neutral pH was achieved and dried (Agrawal et al., 2012, Basu et al., 2015; Dubey et al., 2018; Ali et al., 2018). The de-carbonated samples were introduced into an Elemental Analyzer (Flash EA 2000 HT) through an auto sampler. Through the combustion CO_2 gas was produced and introduced into a Continuous Flow Isotope Ratio Mass Spectrometer (CFIRMS, MAT 253) coupled with

Con-Flow IV interface for isotopic analysis. IAEA CH3 was used to calibrate the reference gas and carbon isotopic data has been reported against VPDB. International standards (IAEA CH3 and CH6) as well as internal standards (Sulfanilamide) were run to check the accuracy for the CO_2 measurements with an external precision of ±0.1‰. Total organic carbon (TOC) was calculated from the peak area obtained from the sum of the integrated m/z 44, 45, and 46 signal measured in the CFIRMS (Fig. 7).

6. Results

6.1. Macrobotanical assemblage

Macrobotanical remains were recovered in samples from both seasons. Undigested seeds and leaves of Poaceae were predominant, with values upto 70% during summer and 50% during winter. The seeds of Chenopodiaceae and Amaranthaceae are also consistently present up to 20% during summer and 30% during winter in the macrobotanical assemblage. Arboreal taxa, especially the invasive species, *Prosopis juliflora* along with *Acacia* pods, leaves, and seeds were also consistently present with values of 10% during summer and increase to 20% during the winter season.

6.2. Pollen and non-pollen palynomorph spectra (Fig. 8)

Poaceae-Cyperaceae-Prosopis-Grewia-Sporormiella-Sordaria-Arcella-Botryococcus assemblage: The dung samples (KS1–KS15) collected during the summer were characterized by the predominance of non-arboreal taxa (50.0%) over arboreal taxa (15.0%). Among non-arboreals, Poaceae are recorded at an average value of 22.0% in the pollen assemblage. The other non-arboreal taxa such as Cyperaceae, Chenopodiaceae, Amaranthaceae, and Malvaceae

are also consistently present with values of 0.5% to 11.3%. Among the non-pollen palynomorphs, thespores of coprophilous fungi, *Sporormiella*, *Podospora*, and *Sordaria* were recorded at 22.0% followed by non-coprophilous fungal spores (10.0%), *Arcella* (1.8%) and *Botryococcus* (1.3%).

Poaceae-Cyperaceae-Chenopodiaceae-Prosopis-Lantana-Sporormiella-Saccobolus-Arcella assemblage: The winter dung samples (KW1–KW15) were also characterized by the dominance of non-arboreal taxa (60.6%) over arboreal taxa (10.6%). Among non-arboreals, Poaceae are present with an average value of 21.0% in the palynoassemblages. Other non-arboreal taxa such as Cyperaceae, Chenopodiaceae, Amaranthaceae, and Malvaceae are also consistently present but lower values of 0.5% to 12.8%. Among the non-pollen palynomorphs, the spores of coprophilous fungi *Sporormiella*, *Saccobolus*, and *Podospora* were most common at 21.2% followed by non-coprophilous fungal spores (6.6%). Both *Thecamoeba* (0.5%) and *Botryococcus* (0.5%) were consistently present at trace values or absent in the palynoassemblages in some samples.

6.3. *Phytoliths spectra*

In summer samples, the Poaceae are dominant in the assemblage across all samples. Among the Poaceae morphotypes, the frequency of grass silica short cells (GSSCs) was 53-69 % of the sample. The GSSCs saddle morphotype was present with values of 17-23% followed by bilobates (10-16%), rondels (8-12%), trapezoids (1-9 %), crosses (5-8%), and long saddles (1-6%). Elongate cells, including dendritic types and psilate types, were present at 1-5% and 1-4% respectively. Various multicell panels were also consistently encountered ranging from 2.5-12%. Among the non-Poaceae/arboreal phytolith types, the cylindric sulcate (tracheary)

morphology predominates with values of 12-20%, followed by acicular hair cells (1-8 %) and stellate hair cells (1- 6%). Cyperaceae morphotypes were recorded at 3-5% (Supplementary Fig. 2).

Similarly, in winter dung samples, the phytoliths of Poaceae predominated at 43-55 %. Among GSSCs morphotype, the saddle type was recorded from 10 to 22%, followed by bilobates (11-16 %), crosses (4-8%), trapezoids (2-8%), rondels (3-6%), and long saddles (2-5%). The elongate dendritic and elongate psilate are also recorded at 3-9% and 2-7% respectively. The cylindric sulcate in arboreal types range from 13-18% followed by stellate hair types (3-9%) and acicular types (2-7%). A Cyperaceae type was also recorded at values of 2-5% (Supplementary Fig. 2).

6.4. Palynofacies spectra

The dung samples studied from the summer season are composed of a high percentage of brown degraded organic matter (DOM) (28-35%) with structured organic matter (SOM) ranging between 25-29%, stellate trichomes (18-20%), grass oxidized tissue (8-10%) and fungal remains (4-6%) respectively. The AOM (3-5%) and pollen/spore (2-3%) occurrence is minimal. It is noted that in the SOM, the tracheid and cuticle vary in equable proportion (Supplementary Fig. 3).

Similarly, the winter dung samples show an enhancement in all the components seen in the summer sample. The SOM has the highest representation of 34-36% followed by grass oxidized tissue (25-30%), brown degraded OM (20-25%), stellate trichome (10-15%), fungal

remains (6-10%), pollen/spore (4-6%), and AOM (3-5%). In winter samples, the tracheids are better represented than the cuticle (Supplementary Fig. 3).

6.5. *FESEM-EDS analysis data*

The data generated from the FESEM-EDS elemental analysis of the summer dung samples recorded that the oxygen (O_2) content/level is 51.65 (weight %), followed by C, 37.3 (weight %), N, 4.42 (weight %), Si, 3.16 (weight %), Ca, 0.84(weight %), Al, 0.78 (weight %), and K, 0.47 (weight %) (Fig. 6a) (Supplementary Table 1).

Similarly, the data recorded from the FESEM-EDS elemental analysis of the samples observed that the oxygen (O_2) content/level is 50.92 (weight %), followed by CK, 38.5 (weight %), N, 4.51 (weight %), Si, 2.89 (weight %), Al, 0.75 (weight %), and K, 0.37 (weight %) (Fig. 6b) (Supplementary Table 2).

6.6. *Stable carbon isotope data*

The average $\delta^{13}C$ values of summer dung samples varies from –15.8‰ to –26.3‰. The TOC values were also recorded from 31.4% to 40.1%, respectively. Similarly, the average $\delta^{13}C$ values of winter dung samples were recorded from –28.1‰ to -30.9‰. The TOC values vary from 37.89% to 44.3% and the TN% recorded from1.1 to 1.91 respectively (Fig. 7), (Supplementary Table 3).

7. Discussion

The macro and microbotanical remains in Indian wild ass dung showed that the Poaceae are the primary food plants based on the dominance of both micro (pollen, phytoliths, and

palynofacies) and macrobotanical remains. However, the recovery of pollen and macrobotanical remains of pods, seeds, and leaves of other families, especially of the Fabaceae, Chenopodiaceae, and Amaranthaceae, is indicative of their importance as food plants of the Indian wild ass. The regular presence of dicot phytoliths was marked in both the summer and winter samples, further indicating the importance of these plants to provide micronutrients. The arboreal taxa produce about twenty times fewer phytoliths than monocots (Albert et al., 2003). The regular presence of the chloridoid morphotype in the phytolith assemblage is indicative of the xerophytic nature in and around the study area. The Chenopodiaceae pollen consistently observed in the dung samples is indicative of the aridity in and around the study areas. Many chenopods utilize C_4 photosynthesis and are important ecologically in saline areas and in cold arid deserts (Pyankov et al., 2000). The presence of Cyperaceae pollen and phytoliths are indicative that despite the arid environment, the presence of water-logged habitat in the study area. The Indian wild ass requires drinking water at least once a day and is dependent on the availability of water bodies (Stubbe et al., 2005; Kaczensky, 2010).

The presence of pollen clumping, especially in Poaceae, Chenopodiaceae, and Amaranthaceae, observed in the palynoassemblage are indicative of a local origin and incorporation into the dung through the direct ingestion of the flowers and inflorescences. The regular recovery of Solanaceae and Apiaceae pollen along with Panicoideae morphotype phytoliths in the assemblage is a strong indicator of the anthropogenic activity in and around the study areas. This observation also indicates that the Indian wild ass is moving up to several kilometers for their food and dispersing into the surrounding human dominated landscape (Fig. 2c). The overall pollen data combining the summer and winter samples is indicative of dry

thorny forest with large open areas under arid-to semi-arid conditions which corresponds with the current vegetation and climate in the region.

The abundance of palynofacies especially, DOM and SOM in the palynofacies assemblage is indicative of the composition of the vegetation and compliments the pollen data. The regular presence of stellate trichomes which are found on leaf surfaces (Wagner et al., 2004) have been observed in the assemblages. In the winter samples, it is observed that the structured OM remains are dominant in the palynofacies assemblages, which could be a supplementary source of high nutrition for the Indian wild ass under the dry condition in the region. There is a lowering of stellate trichomes during winter that may reflect that either the Indian wild ass has moved to some nearby areas where it eats plants with a low content of stellate trichomes or the onset of environmental conditions during winter results in a reduction of stellar trichomes. The amorphous OM remains are appreciably similar to the winter samples which may reflect the environmental situations of the local topography.

Among the fungal spores, the coprophilous fungi, especially *Sporormiella*, *Saccobolus*, and *Podospora* are dominant and consistently represented in both season's samples. The regular presence of *Sporormiella* and *Podospora* in the assemblage may simply reflect the cosmopolitan distribution of these two taxa. The non-coprophilous fungi, *Helminthosporium* and *Bipolaris*, which are pathogens of grassland plants, may have been incorporated in the dung through the ingestion of infected grasses and other herbs. The spores of the mycorrhizal fungi, *Glomus* along with hyphae (Fig. 4. o) are also consistently present in the assemblages and -is indicative of the impact of soil erosion (Van Geel, 1978; Anderson et al., 2011) in the region, resulting in dust settling on the vegetation, which is then ingested by the Indian wild ass with its food.

Determination of the nutrient element content in Indian wild ass dung provides information on the vegetation succession and biogeochemical cycle in arid and semi-arid regions. Plant species depend on different nutrient elements and their concentrations in the soil sediments (Koerselmann and Meuleman, 1996; Aerts and Chapin, 1999; Kroon, 2011). A total of 15 nutrient elements, both macro- and micro (C, N, O, Al, Si, Na, K, Cl, Ca, P, Mg, I, Fe, Mn, and Zn), have been recorded from the studied dung samples (Supplimentary Table 1). Both macro and microelements required in the Indian wild ass diet can be provided inorganically or organically. The average stable carbon isotope analysis value of both summer and winter samples of the Indian wild ass dung is -20.6, which indicates the presence of both C_3 and C_4 plants in their diet (Fig. 5). The stable isotope data complements the micro and macrobotanical remains identified in the dung samples.

7.1. PCA analysis for pollen and non-pollen palynomorphs

The pollen and non-pollen palynomorphs (NPPs) retrieved from the dung samples of Indian wild ass from the Rann of Kachchh during summer and winter were subjected to CANOCO 5.0 and based on response data it was found that the pollen and NPPs are compositional and have a gradient of 0.7 SD units long, hence linear method was recommended, and Principal Component Analysis (PCA) was performed on the present dataset. The variability explained by PCA1 and PCA2 comprises of 80% while the total variability of the first four principal components is 87% (Fig. 5).

The present study shows that during winter the amount of pollen is reduced as indicated by the PCA1. It can be observed that Solanaceae and Amaranthaceae are the highest recorded pollen taxa with the steepest increase of the values for corresponding pollen. These pollen taxa

are closely correlated with other pollen types like *Ziziphus*, Cyperaceae, and Chenopodiaceae and to a certain extent with spores of coprophilous and non-coprophilous fungi like *Sporormiella* and *Alternaria*, respectively.

For the summer record of pollen and non-pollen palynomorphs (NPPs) in PCA1 it can be observed that *Prosopis*, Onagraceae, *Acacia*, *Grewia*, *Lantana*, Malvaceae, Poaceae, and *Ephedra* show the steepest increase of the values for corresponding pollen. These pollen taxa are indicative of enhanced arid conditions and low moisture conditions during any season and reflect very limited aquatic conditions. Consequently they can be said that as food plants they are high in availability in the Rann of Kachchh and hence their pronounced occurrence in the diet of the Indian wild ass in the region. These pollen taxa along with their enhanced occurrence are also correlated in the high probability of their presence in the dung as can be observed with the low angles in the same axis.

In the PCA2 axis, the NPPs with steepest increase are represented by *Botryococcus*, *Glomus*, *Tetraploa*, and *Pleospora* while the pollen and NPPs with increasing arrow heads are Amaranthaceae, *Sordaria*, *Bipolaris*, *Arcella*, *Saccobolus*, and *Helminthosporium*. Similarly, based on their correlation, we find that, these pollen types and NPP's are closely related, and they share a similar type of environmental preference and reflect the Rann of Kachchh environmental settings.

7.2. Indian wild ass dung and its implication for archaeology

Archaeological research on the rise of human civilization in western India has primarily focused on plant domestication (Pokharia et al., 2011, 2017). This has been complemented by studies on the domestication of cattle and sheep based on archaeozoological evidence (Patel,

2009). These traditional approaches can be complemented by the study of modern and fossil dung in relation to the soil sediments of archaeological sites in western India. While the materials found within animal dung are relevant to archaeology (Fig. 2d) the study of this component of a site is still wanting in Indian archaeological research.

. The characteristics of the dung of animal species forms the basis of identification of archaeological dung deposits (Schelvis, 1992) which may represent various human activities on site, such as use of dung as a building material, for fuel and other purposes (Shahack-Gross, 2011). Small equid feces were encountered frequently during excavations in the Chehrabad salt mine in Iran (Askari et al., 2018). Given the potential preservation of equid and other feces at other archaeological sites, this study of Indian wild ass dung can serve as a model for coprolite as well as the soil sediments of the archaeological sites. The presence of spores of coprophilous fungi such as *Sporomiella*, *Podospora*, and *Sordaria* and differences in their relative abundance can result in distinct assemblages in the soil sediments in conjunction with pollen and non-pollen palynomorphs. For example, with an increase in the number of domestic cattle on the landscape, there should be a concurrent increase in dung fungal spores incorporated into the soil (Davis and Shafer, 2006) although there are many complicating factors for proper identification as the spore size varies (swelling or shrinking) due to chemical treatment (Van Geel et al., 2003; Van Geel and Aptroot, 2006; Van Asperen et al., 2021).

The presence of hemiones in archaeological sites may be due to either remains of wild individuals that were hunted, or potentially may be domesticated animals (Noble, 1969; Askari et al., 2018). There is evidence that other subspecies of hemiones such as the Persian onager (*Equus hemionus onager*) were domesticated elsewhere, such as at Chehrabad, an ancient salt mine in northwestern Iran (Askari et al., 2018). There is currently no evidence that the Indian subspecies

or khur was domesticated. However, further study is needed to understand the interactions between past human populations and animals in India, including events such as extinction or domestication. The remains of equids have been reported from the Indus and Harappan archaeological site at Kanmer (5–3.5 ka BP) in the Kachchh District of Gujarat, along with wild and domesticated animals (Goyal et al., 2013). Elsewhere in Gujarat, it has been hypothesized that semi-nomadic pastoralists managed wild plants and herded animals from ca. 7000 BP onwards, culminating in the domestication of several millets and pulses (Garcia-Granero et al., 2016). These types of habitat overlap partially with the current range of the Indian wild ass in Gujarat, and our data may have the potential for helping to better understand the types of habitats used by these past human populations and their interactions with wild herbivores.

These interactions may help contextualize potential remains of wild ass recovered from archaeological sites such as Shikarpur in Gujarat (Thomas et al., 2015) and SohrDamb Nal, Pakistan (Benecke and Neef, 2005). The archaeological site of Loteshwar, located on the margin of a salty waste depression east of the Little Rann of Kachchh have also recorded hemione remains along with other faunal remains, all of which were considered wild species that were hunted in the older Microlithic deposits but hemiones are absent in the later Chalcolithic deposits which primarily contained remains of domesticated species (Patel, 2009).

8. Conclusions

Our multiproxy approach has contributed to understanding the diet of Indian wild ass in the arid-region of Kachchh, Gujarat, India and demonstrates the potential for utilizing these data to aid in examining animal-plant relationships and interpreting the interaction between humans and animals in the archaeological record. Due to the scarcity of water bodies on the landscape

that normally accumulate and preserve pollen it is very difficult to study the modern pollen-vegetation relationship in arid regions. Our study of the dung of the Indian wild ass demonstrates that its pollen data reflects the thorny dry forest under arid condition and could serve as a reliable baseline for the interpretation of palaeovegetation and climate analysis. The consistent presence of *Prosopis juliflora* in the dung of the wild ass supports previous studies (Pasha et al., 2015) that the spread of seeds of this invasive species is facilitated by its inclusion in the Indian wild ass diet. The negative impact by the exotic mesquite on grass availability in the Wild Ass Sanctuary (Sinha et al., 2009) reduces the availability of grasses which as shown here are a primary food source of the Indian wild ass. Consequently, recognition of the negative interaction between these two food sources of the Indian wild ass provides critical information for the management of this endangered species inhabiting a desert ecosystem where resources can fluctuate widely. The stable carbon isotope data will be useful for the characterization of the herbivores coprolites as grazer or browsers in relation to the sedimentary soil in natural and archaeological sites in regional and global level. The analysis of coprolites can also be applied to the identification and differentiation between domesticated and wild herbivores in relation to pastoralism during early human civilization by both their analysis as well as the detailed examination of cultural sediments in western India and its neighboring areas and contribute to a better understanding of the role of domesticated and wild herbivores in the economies of these civilizations.

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Legends

Fig. 1. Location map showing the study area.

Fig. 2. Field photographs a. Close up of *Equus hemionus khur* in its natural habitat, b. A midden dung of *Equus hemionus khur,* c. Practices of crop cultivation by the local people in and around the Little Rann of Katchch, d. A view of domesticated animals during drinking water time in and around the study areas.

Fig. 3. Macrobotanical remains recovered from the *Equus hemionus khur* dung sample.

a. *Panicum* (Poaceae), b. *Dactyloctenum* (Poaceae), c. and d. *Eleusine* (Poaceae), e. Poaceae seed, f. *Chenopodium* seed, g. *Chenopodium* germinating seed, h. and i. Amaranthaceae seed, j. *Celosia* germinating seed, k. Asteraceae seed, l., m. and n. Legume seed, o. Dicot leave.

Fig. 4. Pollen and non-pollen palynomorphs assemblage recovered from the *Equus hemionus khur* dung samples.

a. and b. *Acacia* sp., c. *Grewia* sp., d. *Ephedra* sp., e. Amaranthaceae, f. Chenopodiaceae, g. Malvaceae, h. Apiaceae, i. Solanaceae, j. Poaceae, k. Cyperaceae, l. Onagraceae, m. *Arcella* sp., n. *Botryococcus* sp., o.*Glomus*, p. *Podospora* sp., q. *Sporormiella* sp., r. *Saccobolus*, s. *Pleospora* sp., t. *Bipolaris* sp., u. *Alternaria* sp.

Fig. 5. The PCA analysis of the pollen assemblages of *Equus hemionus khur* dung between summer and winter samples

Fig. 6. FESEM-EDS analysis micrographs in *Equus hemionus khur* dung samples collected from summer (a) and winter (b).

Fig. 7. Stable carbon isotope analysis micrographs in *Equus hemionus khur* dung samples.

Fig. 8. Pollen and fungal spore spectra of the studied *Equus hemionus khur* dung samples.

Declaration of Interest

The authors hereby declare that there is no conflict of interest regarding the manuscript entitled **"Multiproxy analysis on Indian wild ass (*Equus hemionus khur*) dung from Little Rann of Western India and its implications for the palaeoecology and archaeology of arid regions".**

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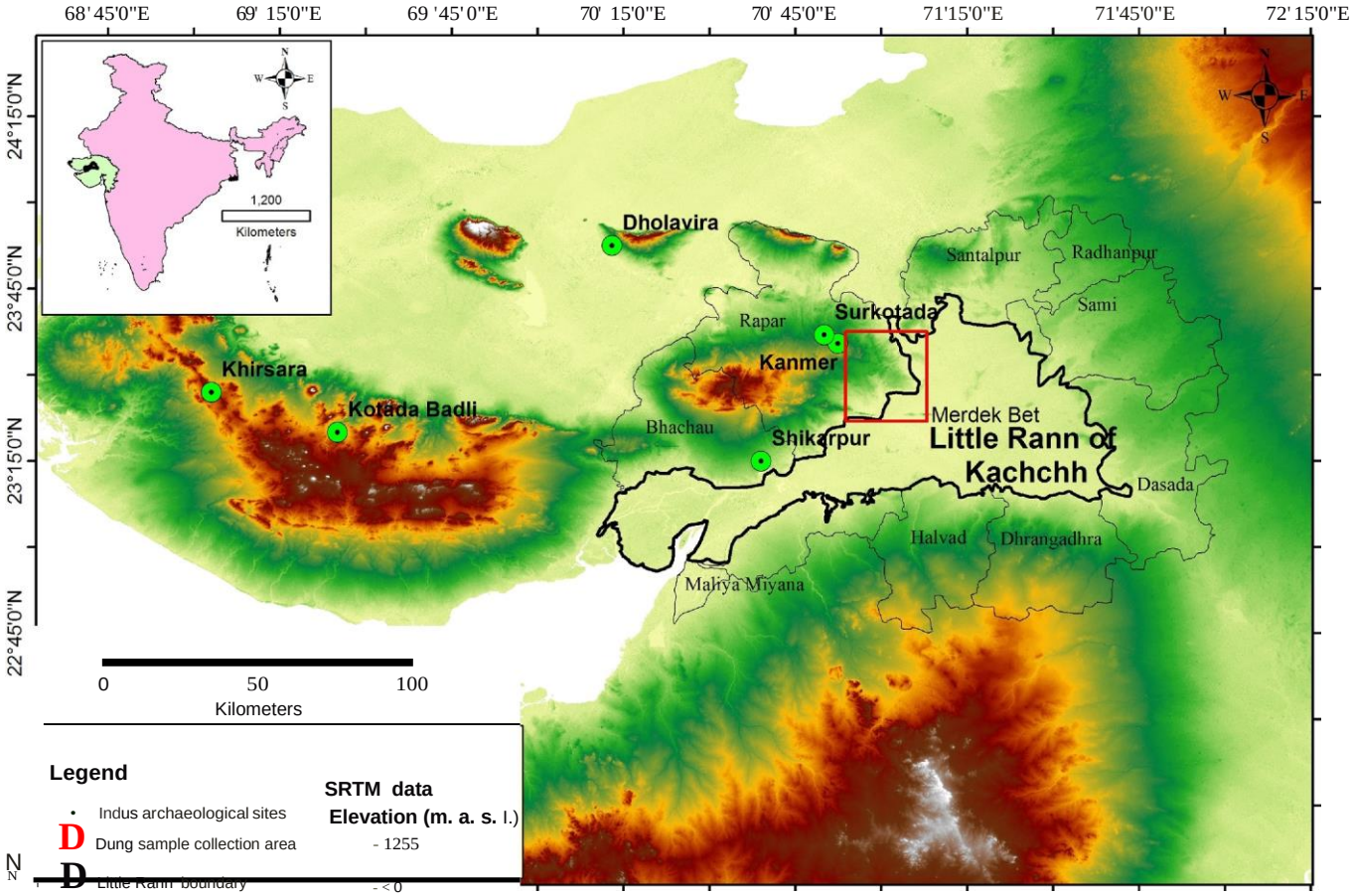
Highlights

- Multiproxy data is documented on the endangered *Equus hemionus khur* dung.
- Pollen data reflected a dry thorny forest under arid-to semi-arid conditions.
- The spore records of coprophilous fungi, especially of *Sporormiella* were discussed.
- Data will be helpful for Palaeoecological and Archaeological studies in arid environment.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Khirsara
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Rapar
Surkotada
Kanmer
Bhachau
Shikarpur
Merdek Bet
Little Rann of Kachchh
Santalpur
Radhanpur
Sami
Dasada
Halvad
Dhrangadhra
Maliya Miyana
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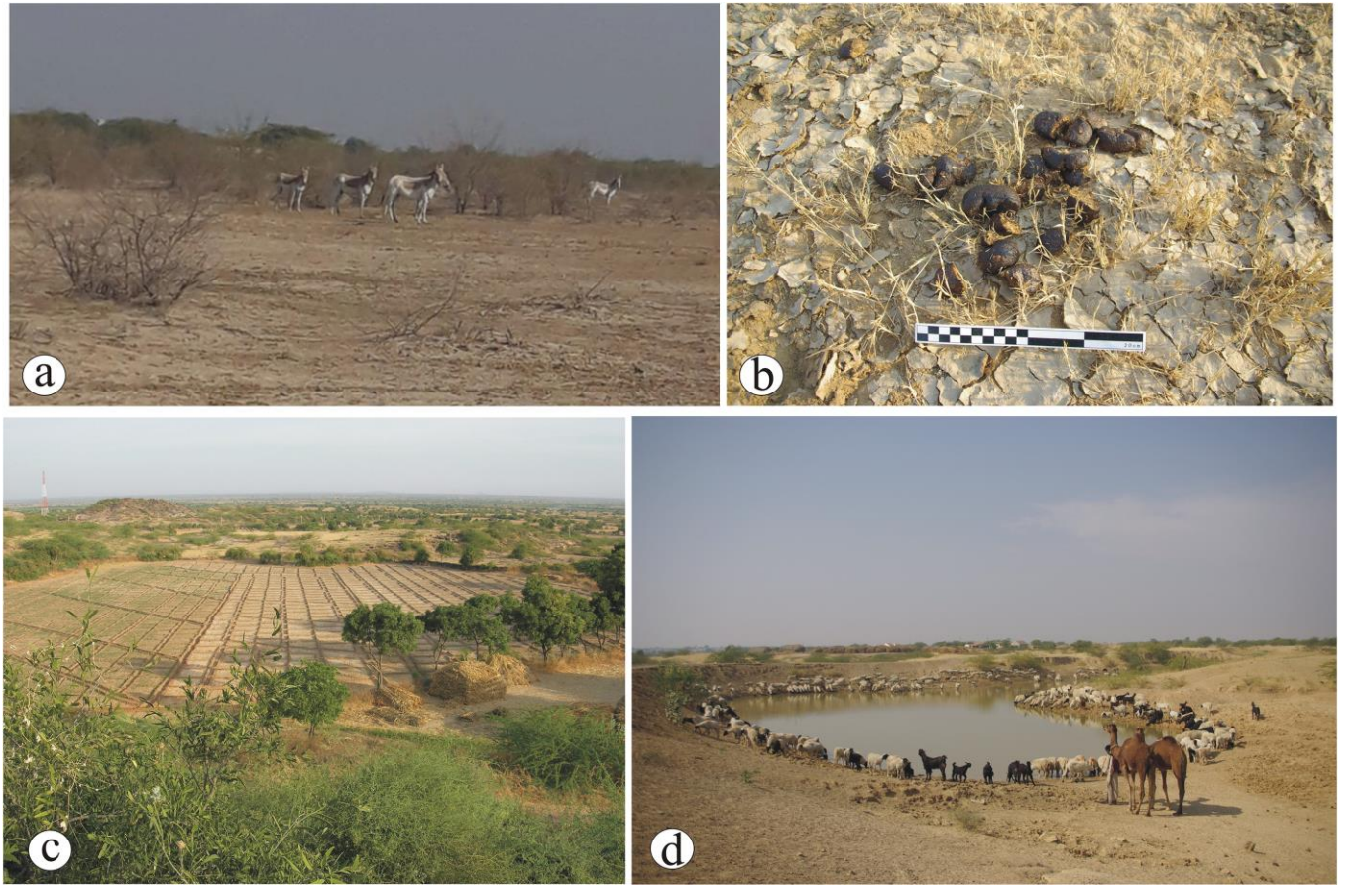
Dung sample collection area
Little Rann boundary
SRTM data
Elevation (m. a. s. l.)
- 1255
- < 0

Figure 1



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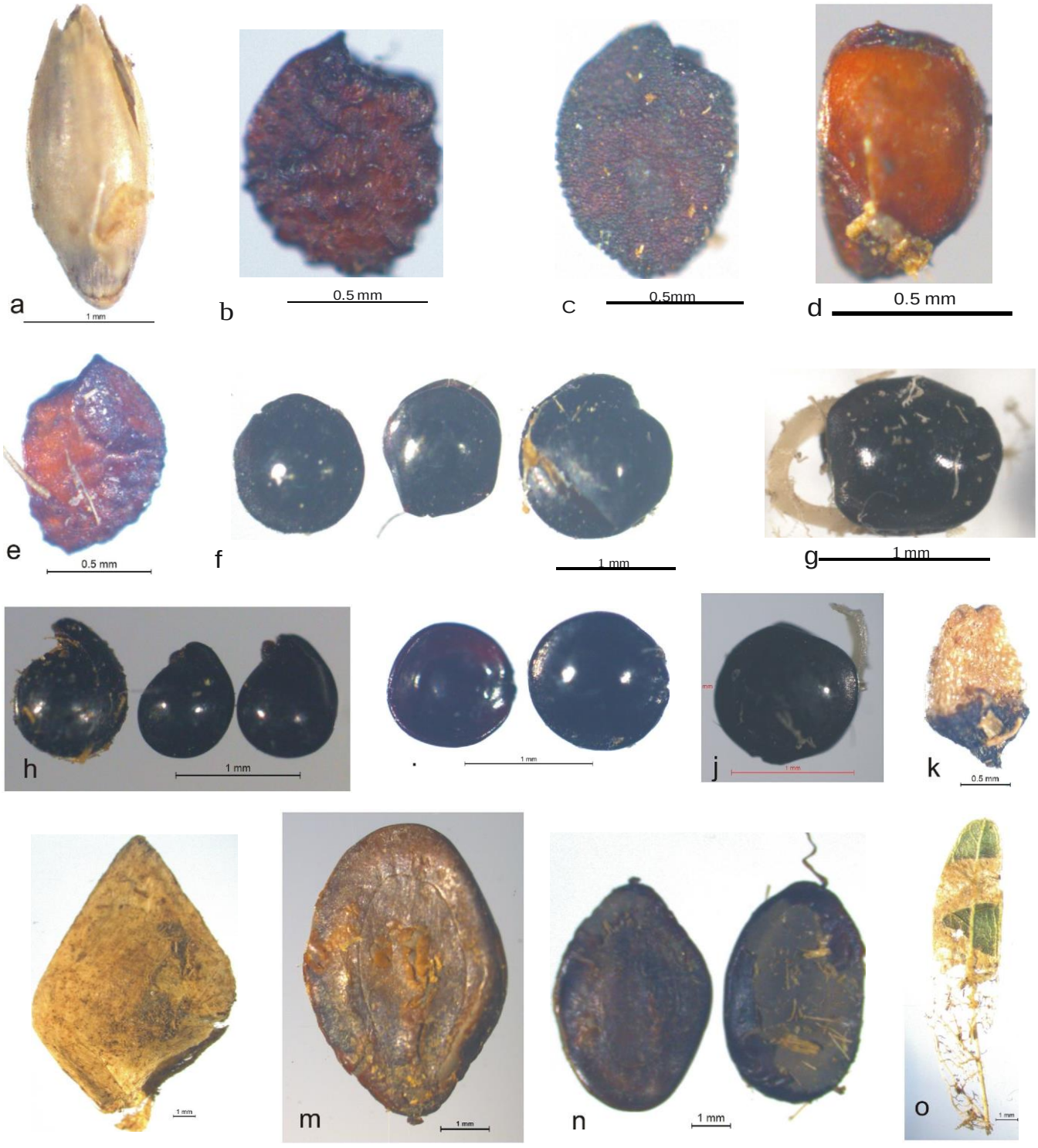
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Figure 3

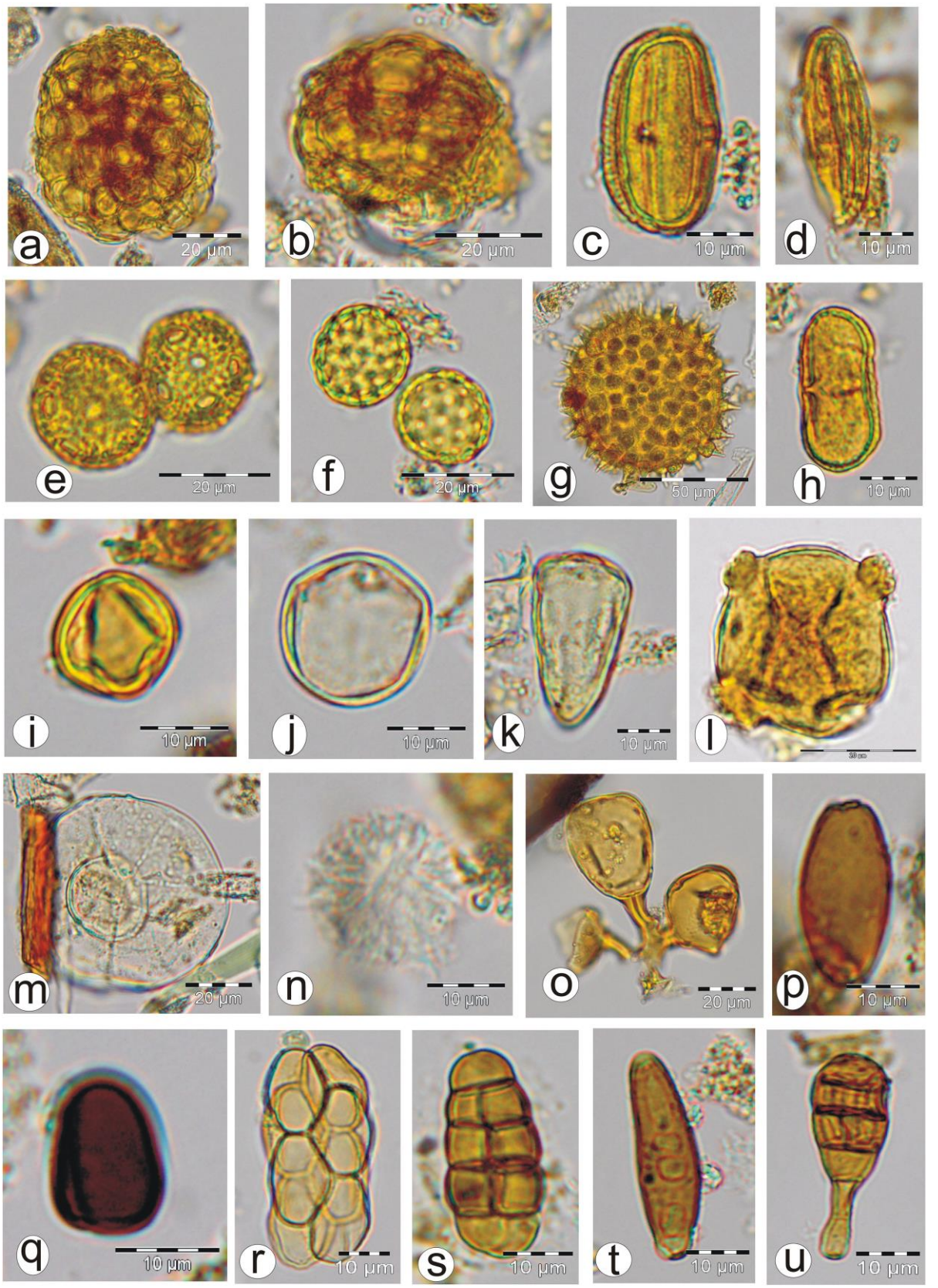
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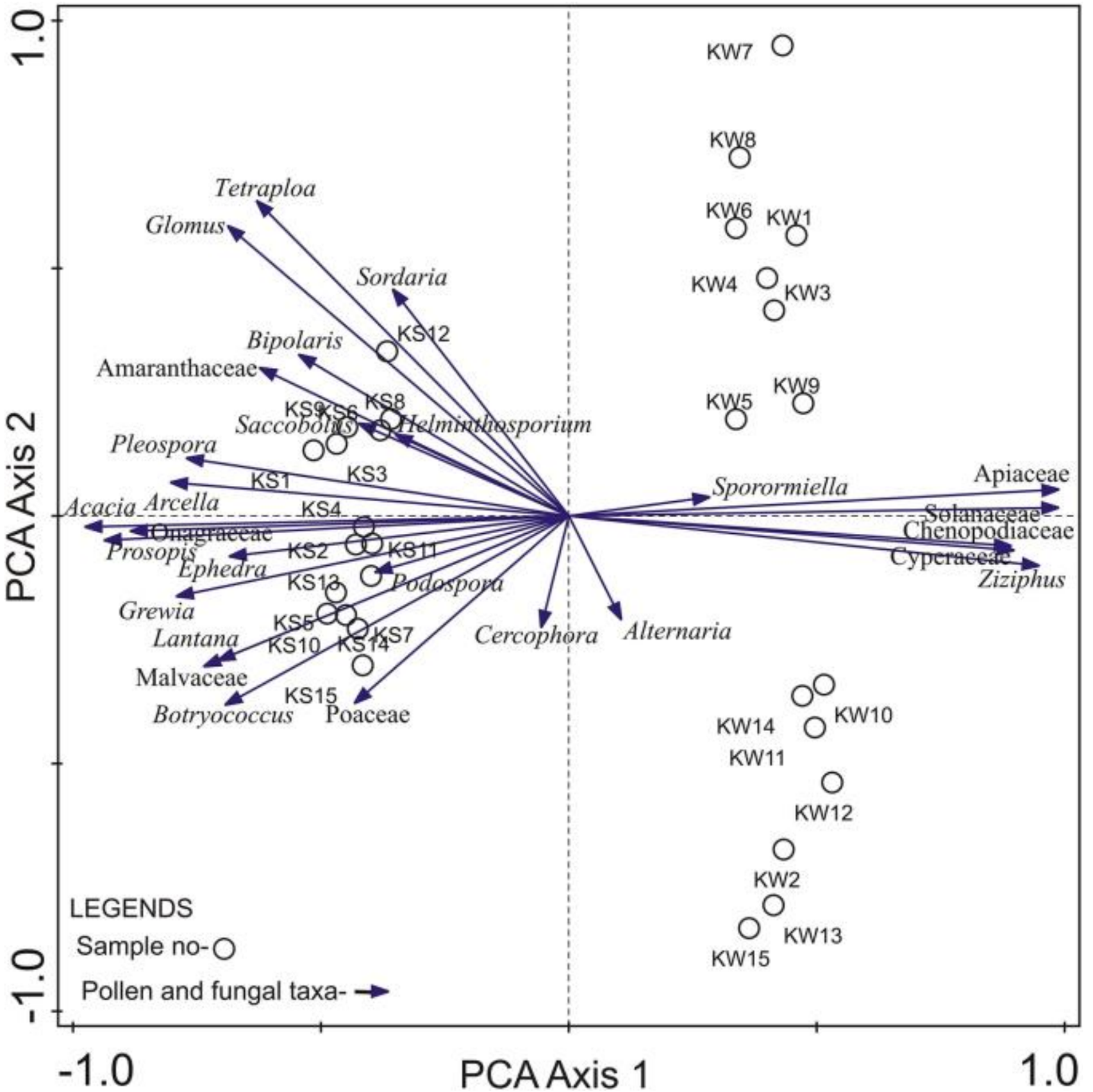
Figure 4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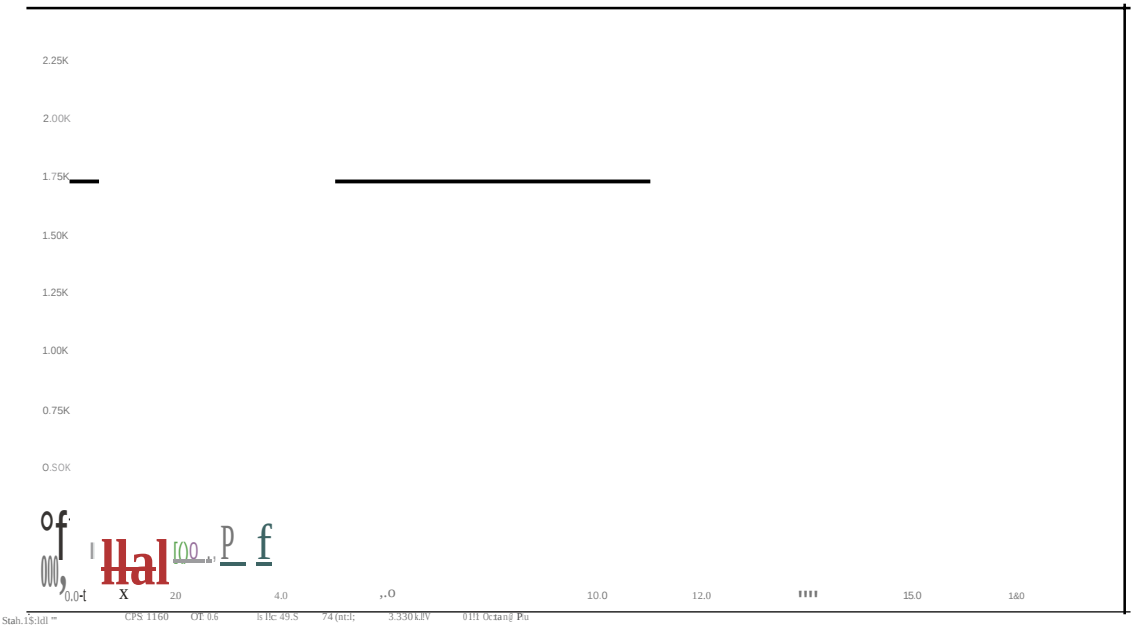
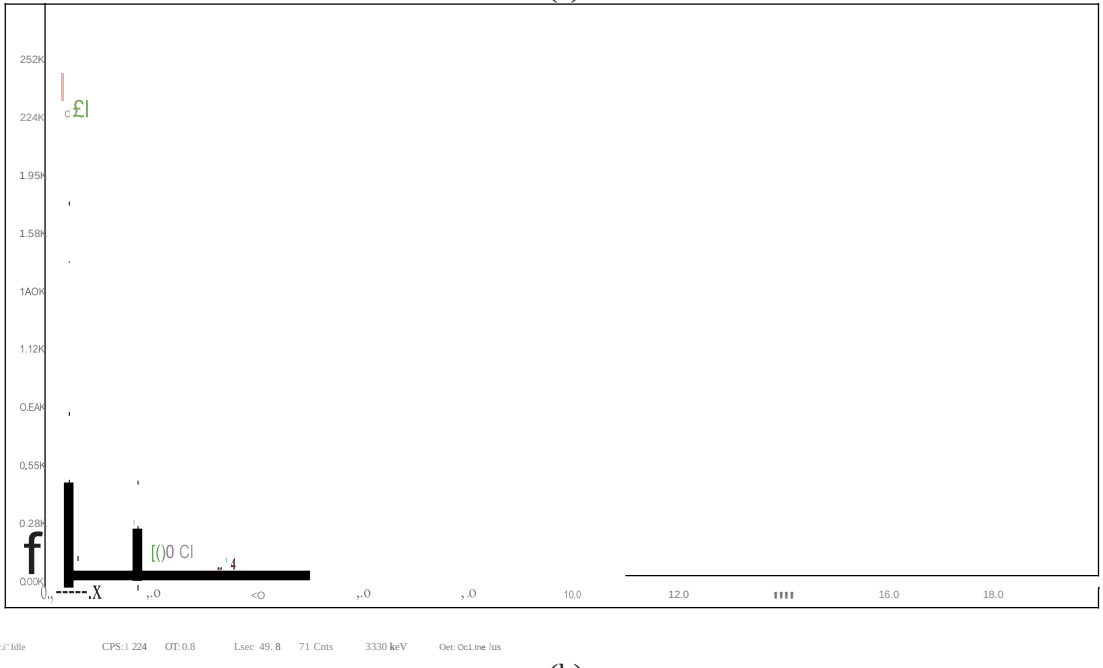
KW15
Tetraploa
Glomus
Sordaria
KS12
Bipolaris
Amaranthaceae
KS9
KS6
KS8
Saccobolus
Helminthosporium
Pleospora
KS1
KS3
Acacia
Arcella
KS4
Onagraceae
Prosopis
KS2
KS11
Ephedra
KS13
Podospora
Grewia
KS5
Lantana
KS10
KS14
KS7
Malvaceae
KS15
Botryococcus
Poaceae
Sporormiella
Apiaceae
Solanaceae
Chenopodiaceae
Cyperaceae
Ziziphus
Cercophora
Alternaria
LEGENDS
Sample no-
Pollen and fungal taxa-

Figure 5



(a)

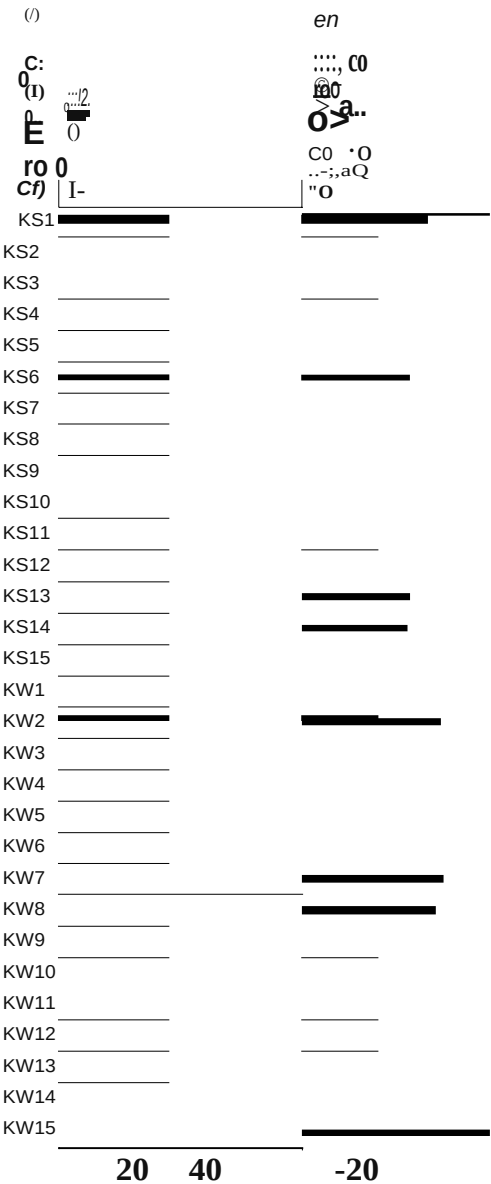


(b)

LEGENDS

X axes: Energy **(Kilo** Electron Volt)
Y axes: Intensities

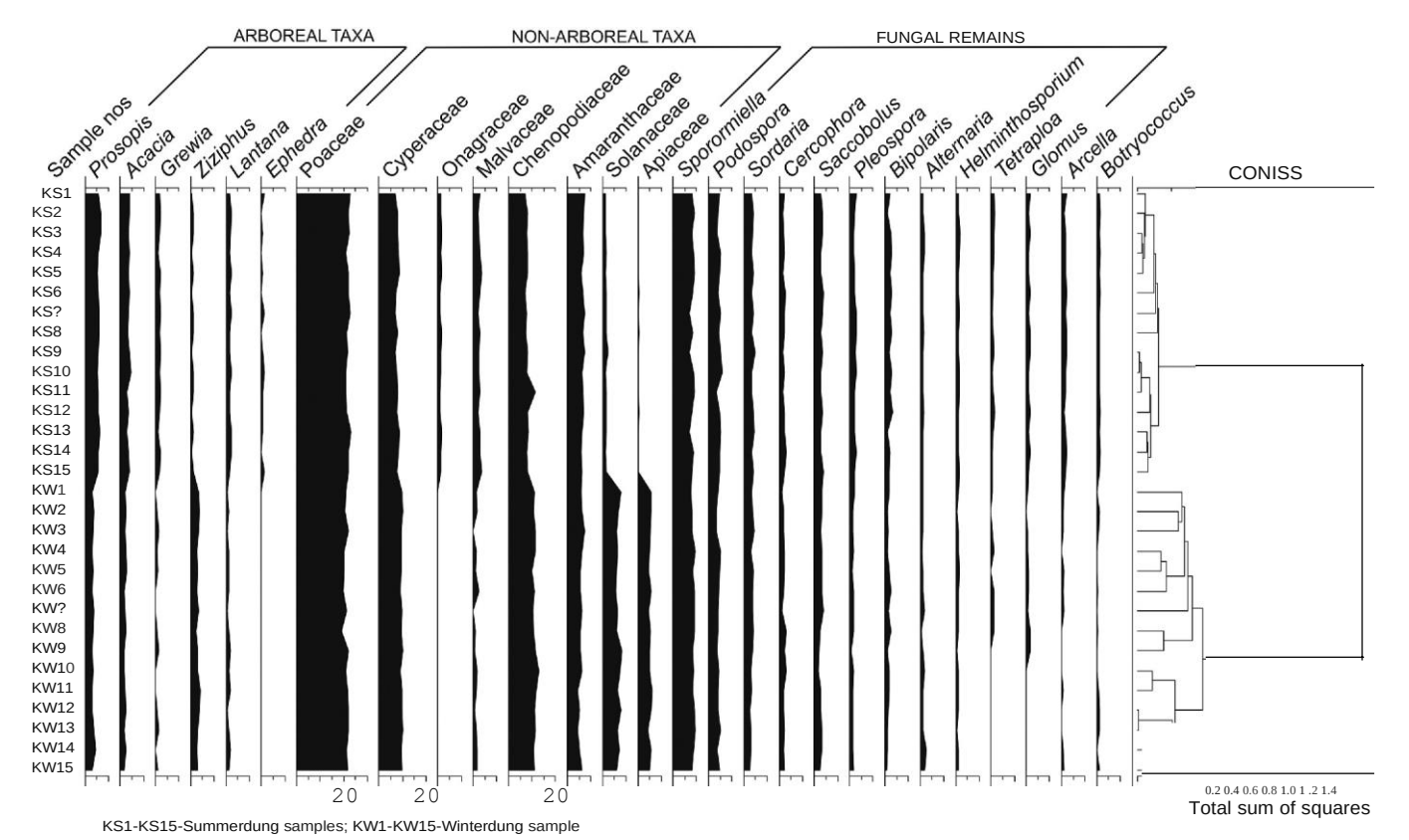
Figure 6



KS1-KS15- Summer dung sample

KW1-KW15- Winter dung sample

Figure 7



KS1-KS15-Summerdung samples; KW1-KW15-Winterdung sample

Figure 8