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Potential of Hairs as a Decisive tool in Forensic Palynological investigations: First experimental study from India

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Abstract

The present study was performed to assess the feasibility of spores, pollen grains and other microscopic entities isolated from human hairs in associating suspects and objects with a particular location in Indian context. 19 hair samples of volunteers of different places of West Bengal viz., Baruipur (with tropical evergreen vegetation), Uttar Kashiabad, Pakhiralaya, Gosaba (with mangrove vegetation) and Ravangla of Sikkim (with high altitude vegetation) were collected. Pollen grains of Aegiceras sp., Kandelia sp., Sonneratia apetala, Acanthus ilicifolius, Justicia sp., Phoenix sp., Clerodendron sp., Avicennia sp., Lathyrus sp., Leucas sp., Commelina sp., Rhizophora sp. were recovered from the hair samples of Pakhiralaya, Gosaba and Uttar Kashiabad of the Indian Sundarbans. The recovered palynotaxa from Baruipur were Cocos nucifera, Borassus flabellifer, Peltophorum sp., Hygrophila sp., Chrozophora sp., Phyla nodiflora, Helianthus annuus, Cleome sp., Cheno-ams, Poaceae and Litchi chinensis. Hair samples from Ravangla have yielded Trifolium sp., Pinus sp., Duabanga sp., Rhododendron sp., Erigeron sp., Alnus nepalensis and Cryptomeria japonica. Correspondence analysis (CA) data helped to ascertain that the recovered palynmorphs from hair samples were from three distinct phytogeographical locations. Thus, through palynological study of human hairs, places of interest can be located and trace evidence can be provided to link people and objects with crime scenes in India.

Keywords: Hairs, palynomorph assemblages, forensic palynology, India.

Introduction

Use of spores and pollen grains to solve legal problems is well established. Pollen grains have been used frequently in solving forensic cases in Sweden[1], New Zealand[2-17], Australia[18,19], the United Kingdom[20-24] and the United States of America[25-30]. However, in India till date their use has not even been considered as associative evidence. Generally, forensic palynology has already been used in solving criminal cases based on associated pollen/spore assemblages from soil, clothes, and footwear of a person (suspect/victim) found at a crime scene. Its main forensic value lies in providing associative evidence that may assist in proving or disproving a link between people or objects with places or with other people or objects.

Woven cloth, woolen blankets, ropes, clothing and fur all make excellent traps for pollen grains and spores which are stuck into their tiny interwoven fibres[31]. When wind blows through hairs, pollen in the wind becomes trapped in the open spaces between individual hair strands. Human hairs are 50-70 µm in diameter and the use of various hair sprays, natural oils, and tonics makes hair surfaces sticky and provides a better trap for pollen and spores. Hairs exhibit Piezoelectricity and Pyroelectricity by attracting particulates through electrostatic forces[32].

Hairs grow from hair follicles, the bulb (root) of the hair is contained within the follicle, the shaft of the hair connects with the root and the shaft has three parts: the cuticle, the cortex, and the medulla, the shaft ends with the tip of the hair. Generally, criminalists attempt to match the color, length, and diameter of the hair, presence of medullae, and the pigment granules' shape, size, and distribution are important comparable characteristics. Wherever a criminal steps, whatever he touches and whatever crime he commits, he will leave unconsciously 'marks' that will serve as a silent witness against him. During an offence, the hairs of an offender/victim may have physical contact with plants or soil and palynomorph assemblages adhered to hairs thus may provide forensic evidence. Animal fur used as car seat cover, hats, bags, felts or clothes can also be very useful for study of pollen grains in any legal issue[33].

Although it is known that hairs are good traps for pollen grains but experimental database as well as case study based information are meager. Thus, the objective of the present study was to check the feasibility of palynomorphs (pollen grains, spores and other bio particles) recovered from hairs in associating suspects/victims and objects with a particular location in India.

Material and Methods

19 human hair samples were collected from Baruipur (22°22' N, 88°26' E), Uttar Kashiabad (21°53' N, 88°21'E), Pakhiralaya (21°50' N, 88°08' E) and Gosaba (22°10' N, 88°49' E) of South 24 Parganas, West Bengal and also from Ravangla (27° 18' N, 88° 21' E) of Sikkim during April - May, 2015. Details of the collected hair samples are presented in table-1.

Table-1
Details of collected hair samples for palynological investigation from different parts of West Bengal and Sikkim, India

HB -1	Baruipur, South 24 parganas
HB -2	Baruipur, South 24 parganas
HB -3	Baruipur, South 24 parganas
HB -4	Baruipur, South 24 parganas
HR -1	Ravangla, Sikkim
HR -2	Ravangla, Sikkim
HR -3	Ravangla, Sikkim
HU -1	Uttar Kashiabad, South 24 parganas
HU -2	Uttar Kashiabad, South 24 parganas
HU -3	Uttar Kashiabad, South 24 parganas
HU -4	Uttar Kashiabad, South 24 parganas
HP -1	Pakhiralaya, South 24 parganas
HP -2	Pakhiralaya, South 24 parganas
HP -3	Pakhiralaya, South 24 parganas
HP -4	Pakhiralaya, South 24 parganas
HG -1	Gosaba, South 24 parganas
HG -2	Gosaba, South 24 parganas
HG -3	Gosaba, South 24 parganas
HG -4	Gosaba, South 24 parganas

Samples were collected in sterile plastic bags and then analyzed in the laboratory using the method of Horrocks[11] (figure-1). The electrostatic forces holding pollen and other particulates onto hair are easily discharged by washing in hot and diluted detergent solution. For extraction of palynomorphs from hairs, a volume of detergent solution was placed in a clean beaker. Samples were then immersed, agitated and rubbed vigorously for 60 minutes and the residues were placed into 10 ml test tubes and centrifuged at 3000 r.p.m for 5-6 minutes and repeated twice using distilled water. 10% potassium hydroxide (KOH) solution was added to the residue, warmed at 70°C for 10-15 minutes and stirred occasionally. This step breaks up the matrix (deflocculation) and dissolves humic materials, producing a dark brown solution. The residue was washed again using distilled water by centrifugation and decantation. Next, 0.1 M solution of sodium pyrophosphate was added, stirred and placed in boiling (100°C) water bath for 10-20 minutes, centrifuged for 5 minutes and then decanted. The process was repeated twice. Distilled water was then added, stirred, centrifuged and finally decanted. This step is usually required only for soil samples in order to remove clay (fine mineral particles, mainly silica) as a high concentration of clay on prepared slides causes cloudiness and hinders pollen identification. After that 10% hydrochloric acid (HCl) was added to samples and kept for 24 hours. These were then stirred, centrifuged using distilled water at 3000 rpm for 5-6 minutes and finally the supernatant was decanted. The process was repeated twice. The samples were then treated with 40% hydrofluoric acid. Hydrofluoric acid was added and placed in a boiling (100°C) water bath for 15-20 mins. Distilled water was then added, stirred, centrifuged and finally decanted. Finally, residue was treated with acetolysis mixture (acetic anhydride and concentrated sulfuric acid in v/v = 9:1[1,3,11,34]. The solution was placed in a boiling (100°C) water bath for 4.0-4.5 mins., centrifuged and finally decanted. Slides were prepared using the method of Horrocks[11]. A minimum of 200-250 palynomorphs were counted from each of the samples[17]. Light photomicrography of the different morphotypes was carried out using Zeiss Axioskop-2 microscope with an image analyzing kit. Identification of different spores and pollen grains was carried out through consultation of reference slides prepared and stored in the Palaeobotany-Palynology Laboratory, Department of Botany, and University of Calcutta. Published literature was also consulted during identification of certain taxa. Correspondence Analysis (CA) were performed with the palynological data from 19 hair samples of volunteers collected from different places of West Bengal and Sikkim as mentioned. Ultra structural details of collected hairs and some recovered pollen grains have been studied and photographed by Scanning Electron Microscope (figure-1). Collected samples, macerated samples and slides are deposited in the repository of Palaeobotany- Palynology Laboratory, Department of Botany, University of Calcutta.

Results and Discussion

Pollen grains of *Cocos nucifera* L. (Arecaceae), *Borassus flabellifer* L. (Arecaceae), *Peltophorum* sp. (Fabaceae), *Hygrophila* sp. (Acanthaceae), *Cleome* sp. (Capparidaceae), *Litchi chinensis* Sonn. Mill. (Sapindaceae), *Helianthus annuus* L. (Asteraceae), *Phyla nodiflora* (L.) Greene (Verbenaceae), *Chrozophora* sp. (Euphorbiaceae), *Psidium guajava* L. (Myrtaceae), Poaceae, *Terminalia* sp. (Combretaceae), Caryophyllaceae, *Aegiceras* sp. (Myrsinaceae), *Kandelia* sp. (Rhizophoraceae), Cheno-ams, *Avicennia* sp. (Avicenniaceae), *Bruguiera* sp. (Rhizophoraceae), *Lathyrus* sp. (Fabaceae), *Commelina* sp. (Commelinaceae), *Rhizophora* sp. (Rhizophoraceae), *Heliotropium* sp. (Boraginaceae), *Croton* sp. (Euphorbiaceae), *Sonneratia apetala* Buch.-Ham (Sonneratiaceae), *Tridax* sp. (Asteraceae) and *Rungia* sp. (Acanthaceae) were retrieved from the hair samples from Uttar Kashiabad, Pakhiralaya and Gosaba regions of South 24 parganas of West Bengal. Interestingly pollen grains of *Aegiceras* sp. (Myrsinaceae), *Kandelia* sp. (Rhizophoraceae), *Avicennia* sp., *Bruguiera* sp., *Rhizophora* sp. and *Sonneratia apetala* were recovered in higher frequency than those of others and representing a mangrove swampy vegetation (figure-1, 2).

From Baruipur area of West Bengal, pollen grains of *Cocos nucifera* L. (Arecaceae), *Borassus flabellifer* L. (Arecaceae), *Peltophorum* sp., *Hygrophila*, *Cleome* sp., *Litchi chinensis*, *Helianthus annuus*, *Phyla nodiflora*, *Chrozophora* sp., *Psidium guajava*, Poaceae, *Terminalia* sp., Cheno-ams, *Lathyrus* sp., *Commelina* sp., *Heliotropium* sp., *Croton* sp., *Tridax* sp., *Rungia* sp. were recovered. These are commonly growing mesophytic elements of tropical evergreen forest (figure-2).

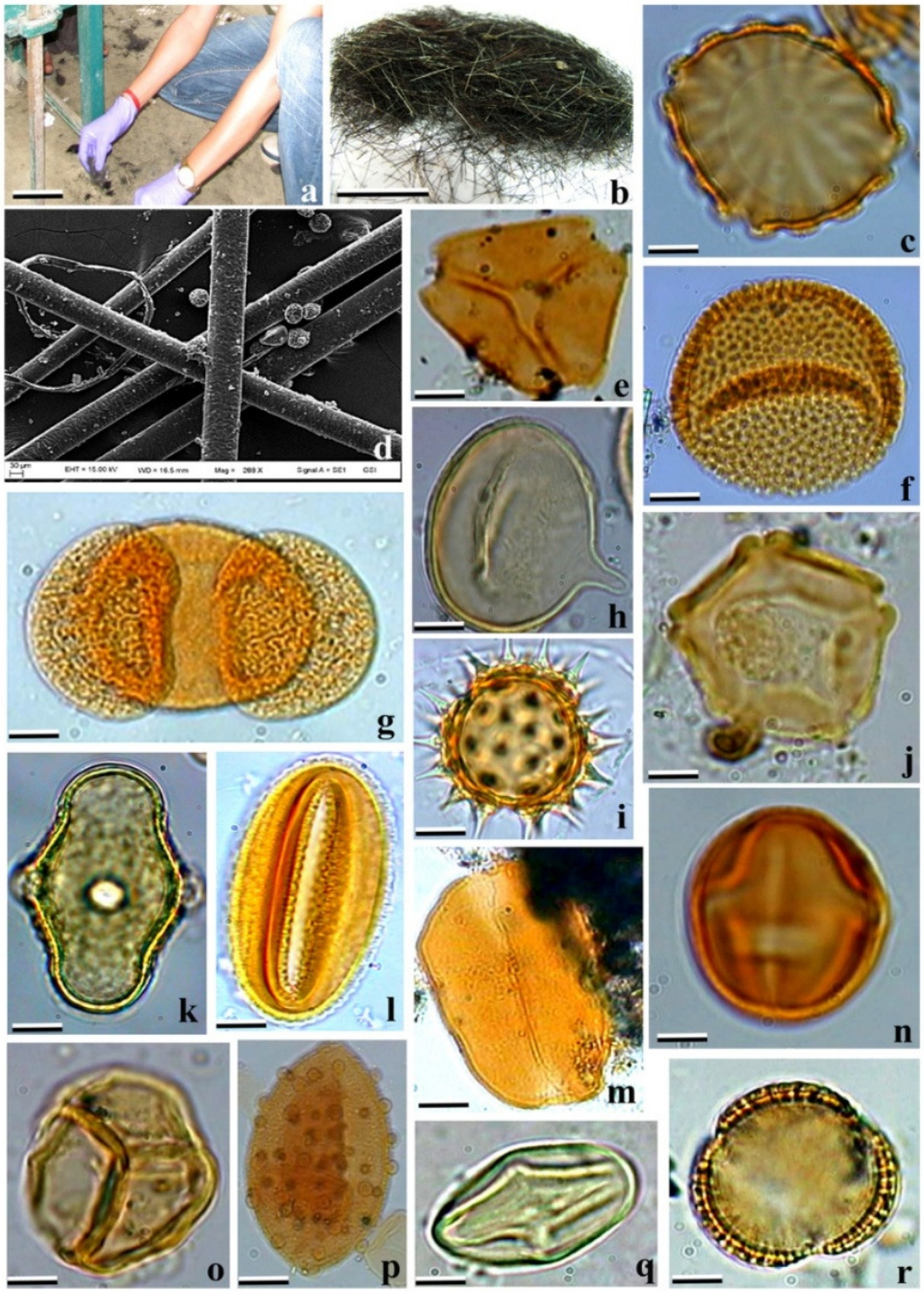

Figure-1
a. Diagram showing the collection of hair samples from volunteers in Baruipur, West Bengal; b. Collected hair samples; c. Pollen grain of *Hygrophila* sp.; d. Scanning electron micrograph of collected hair sample showing attached pollen grains; Pollen grains of :- e. *Syzygium* sp.; f. *Croton* sp.; g. *Pinus* sp.; h. *Cryptomeria japonica*; i, *Tithonia* sp.; j. *Alnus nepalensis*; k. *Sonneretia apetala*; l. *Acanthus ilicifolius*; m. *Cocos nucifera*; n. *Aegiceras* sp.; o. *Rhododendron* sp.; p. *Borassus flabellifer*; q. *Trifolium* sp.; r. *Avicennia* sp. [Scale bar = 5 µm (a = 6 inches, b = 1 inch; g, p = 10 µm)]

Pollen grains of *Trifolium* sp., *Athyrium* sp., *Pyrrosia* sp., *Alnus nepalensis*, *Tithonia* sp., *Arthromeris* sp., *Primula* sp., *Cryptomeria japonica*, *Erigeron* sp., *Duabanga* sp., Poaceae, *Rhododendron* sp., *Selaginella* sp., *Pinus* sp., *Pteris* sp., *Terminalia* sp, Caryophyllaceae and Cheno-ams were found in the hair samples from Ravangla of Sikkim, India showing the high altitude vegetation zones ranging from sub-alpine to tropical wet forest types (figure-2).

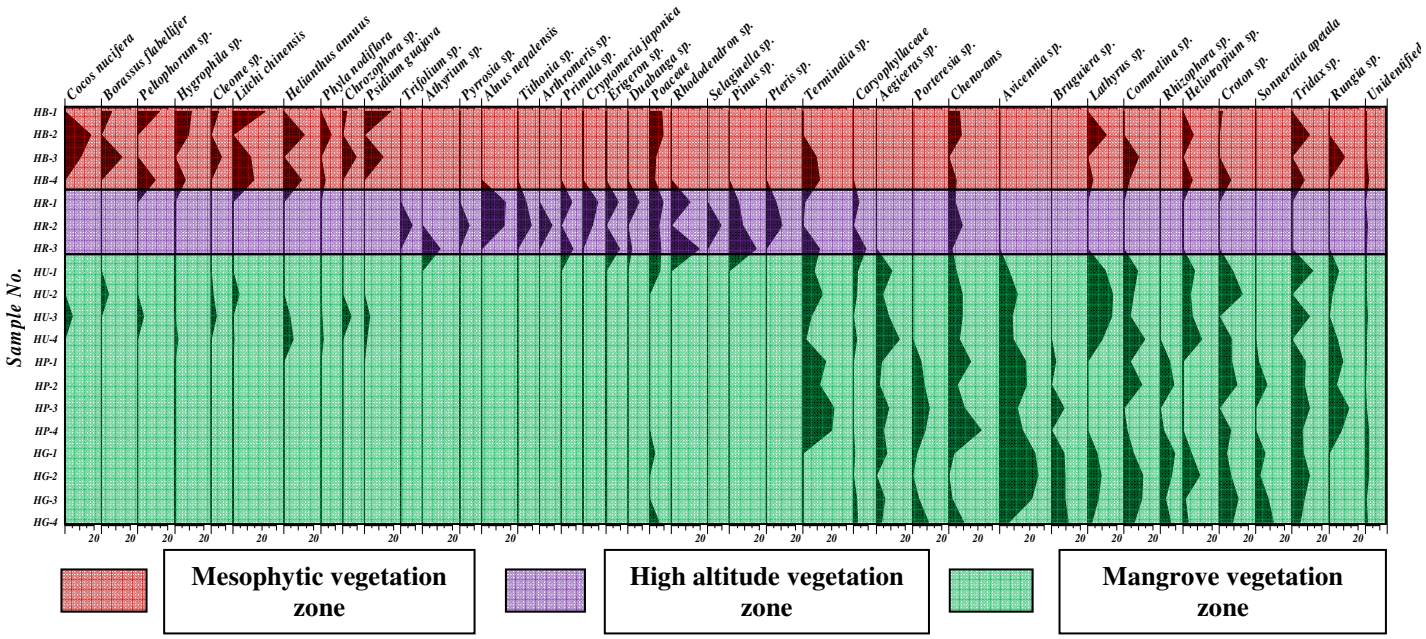

Figure-2
Frequency distribution (%) of occurrence of palynotaxa from hair samples of West Bengal (Baruipur, Uttar Kashiabad, Pakhiralaya and Gosaba) and Sikkim (Ra-vangla) of India

The specific environment in which an organism lives is called a habitat. Plants grow with other plants in any forest types. The communities in which plants grow are governed by soil, temperature, wind, moisture, altitude, latitude and longitude. West Bengal is a state having various types of phytogeographical regions ranging from high altitude temperate to sub-tropical forest of Darjeeling, deltaic halophytic mangrove forests of the Sundarbans and tropical evergreen forests in many of the districts like Murshidabad, Nadia, North 24 parganas, South 24 parganas, Kolkata, Howrah etc. Sikkim is characterized by the high altitude vegetation region ranging from sub-alpine, temperate coniferous, tropical moist, tropical semi deciduous and tropical wet forest types. Results of present study clearly indicate that pollen grains recovered from hair samples are well in conformity with the vegetation of the sampling sites and provides information of local vegetation types and climatic conditions[35].

For comparing associations containing counts of taxa or counted taxa across associations, CA is the more appropriate analysis. Here, CA was applied to the percentage frequency data of the 41 palynotaxa retrieved from 19 hair samples collected from Baruipur, Uttar Kashiabad, Pakhiralaya and Gosaba of West Bengal and Ravangla of Sikkim, India using the statistical program Statistica 6 (figure-3). In the CA coordinate biplot with variance of 27.14% and 18.36% for axis 1 and 2 respectively, 41 types of palynotaxa and 19 hair samples from three distinct clusters (figure-3). Hair samples of Baruipur (HB-1, HB-2, HB-3, HB-4) lie in a single cluster with pollen grains of *Cocos nucifera*, *Borassus flabellifer*, *Peltophorum* sp., *Hygrophila* sp., *Cleome* sp., *Litchi chinensis*, *Helianthus annuus*, *Phyla nodiflora*, *Chrozophora* sp. and *Psidium guajava*. Hair samples from Uttar Kashiabad (HU-1, HU-2, HU-3, HU-4), Pakhiralaya (HP-1, HP-2, HP-3, HP-4) and Gosaba (HG-1, HG-2, HG-3, HG-4) were found in another different cluster with pollen grains of *Aegiceras* sp., *Kandelia* sp., Cheno-ams, *Avicennia* sp., *Bruguiera* sp, *Lathyrus* sp., *Commelina* sp., *Rhizophora* sp., *Heliotropium* sp., *Croton* sp., *Sonneratia apetala* , *Tridax* sp. and *Rungia* sp.

Third cluster shows close association of hair samples of Ravangla (HR-1, HR-2, HR-3) of Sikkim with pollen grains of *Trifolium* sp., *Athyrium* sp., *Pyrrosia* sp., *Alnus nepalensis*, *Tithonia* sp., *Arthromeris* sp., *Primula* sp., *Cryptomeria japonica*, *Erigeron* sp., *Duabanga* sp., Poaceae, *Rhododendron* sp., *Selaginella* sp., *Pinus* sp., *Pteris* sp. and *Terminalia* sp. These three clusters in the correspondence bilpot helped to discriminate three different environmental zones of study area by their palynological composition i.e., Baruipur having a tropical evergreen vegetation type and the pollen grains (*Cocos nucifera*, *Borassus flabellifer*, *Peltophorum* sp., *Hygrophila* sp., *Cleome* sp., *Litchi chinensis*, *Helianthus annuus*, *Phyla nodiflora*, *Chrozophora* sp. and *Psidium guajava*) recovered from the hairs of Baruipur also fall under the same vegetation type. Ravangla of Sikkim is characterized by sub-alpine to sub-tropical deciduous moist vegetation type and the pollen grains (*Trifolium* sp., *Athyrium* sp., *Pyrrosia* sp. *a*, *Alnus nepalensis*, *Tithonia* sp., *Arthromeris* sp., *Primula* sp., *Cryptomeria japonica*, *Erigeron* sp., *Duabanga* sp. *a*, Poaceae, *Rhododendron* sp., *Selaginella* sp., *Pinus* sp., *Pteris* sp. and *Terminalia* sp.) retrieved from the hair samples of Ravangla also suggests the similar vegetation type. On the other hand, forest

type of Uttar Kashiabad, Pakhiralaya and Gosaba of South 24 Parganas of West Bengal ranges from the tropical evergreen mesophytic to mangrove swampy halophytic vegetation. Pollen grains (*Aegiceras* sp., *Kandelia* sp., Cheno-ams, *Avicennia* sp., *Bruguiera* sp., *Lathyrus* sp., *Commelina* sp., *Rhizophora* sp., *Heliotropium* sp., *Croton* sp., *Sonneratia apetala*, *Tridax* sp. and *Rungia* sp.) recorded from hairs of Uttar Kashiabad, Pakhiralaya and Gosaba regions are also included in the same vegetation type[36,37].

Conclusion

Present study suggests that hairs may act as excellent traps for pollen grains, spores and other particulates and thus may be considered for their potential use as a forensic tool in legal cases. It not only proves hairs as good pollen trap but also demonstrates that it is possible to prove or disprove the alibis of suspect/victim from different vegetation zones as each region has a unique pollen print and thus pollen grains from hairs might be useful to solve a crime mystery.

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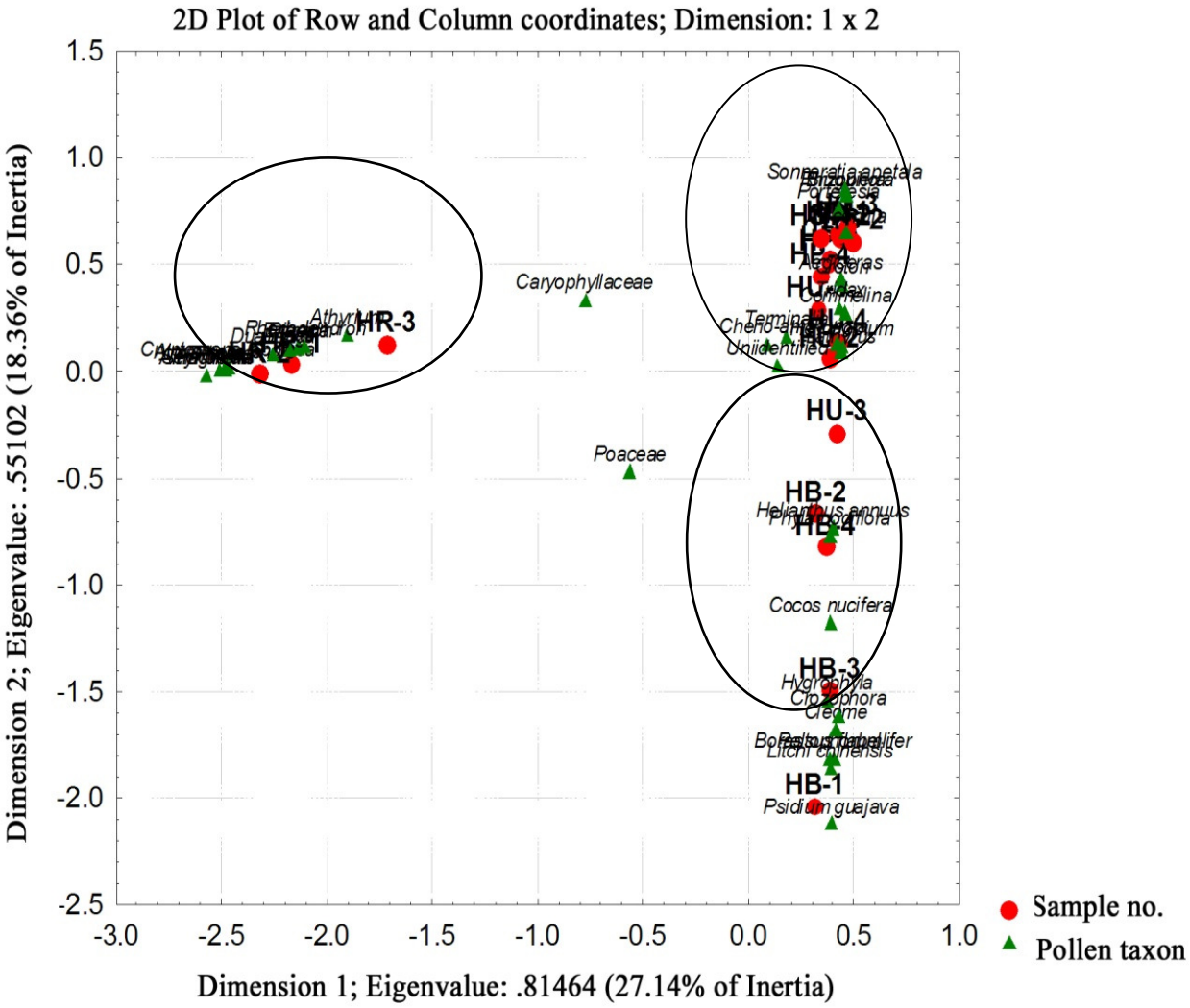

Figure-3
Biplot showing the results of Correspondence analysis of hair samples and recovered palynotaxa

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