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POLLEN MORPHOLOGY OF THE SUBFAMILY ARECOIDEAE GRIFF. (FAMILY-ARECACEAE) FROM PAKISTAN AND KASHMIR

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Abstract

Pollen morphological characters are potentially informative in the systematic of monocotyledons including (Arecaceae), at supra and infra familial level. The pollen morphology of 8 species (subfamily Arecoideae) clearly shows that the qualitative pollen characters (such as aperture, exine pattern and shape) are found to be taxonomically important as compared to the quantitative characters (such as size of the grain and exine thickness).Considerable pollen variations have been found within the subfamily Arecoideae with regard to size, shape, aperture type and exine pattern. For instance, 7 out of 8 species have monosulcate pollen (i.e., 87.5%) and a remaining species (viz., *Elaeis guineensis* Jacq.) have trichotomosulcate pollen. The pollen are frequently elliptical, but in some cases rounded-triangular pollen were also found. Similarly, there is a great diversity in exine pattern such as punctate, reticulate, vermiculate, spinose, rugulate and perforate or combination of these types have also been found, but the most predominant pattern is the reticulate type.

On the basis of pollen aperture, size and exine pattern, four pollen types have been recognized such as *Areca*-type, *Dypsis*-type, *Cocos*-type and *Elaies*-type. The data obtained from the palynological studies have been analyzed by Agglomerative cluster analysis choosing the Euclidean distance and Ward's method for a group linkage method. The objective of the present work is to classify studied taxa on the basis of pollen characters and to quantify the species relationships representing the subfamily Arecoideae based on numerical techniques.

Key words: Pollen, Areceae Drude, Cocoeae Mart. and Roystoneae Cook.

Introduction

The subfamily Arecoideae Griff., is the largest and most diverse among the five subfamilies recognized in the family Arecaceae, consists of 14 tribes and 14 sub-tribes, from which the tribe Areceae Drude is the largest tribe (Dransfield *et al.,* 2008; Baker *et al.*, 2011). About 60% of palm genera (113 out of 186) and 54% of species (c.1300 out of c.2400) representing the subfamily Arecoideae, extend equally in the Americas and Southeast Asia to Western Pacific regions. The members of arecoid palms are widespread in the tropics and sub-tropics regions (mainly in rain forest) and, to a lesser extent, in seasonally dry habitats. The monophyly of subfamily Arecoideae as circumscribed in the current classification (Dransfield *et al*., 2005, 2008) is strongly supported by a substantial body of phylogenetic evidence (Uhl & Dransfield, 1987; Asmussen *et al*., 2000, 2006; Asmussen & Chase, 2001; Lewis & Doyle, 2002; Roncal *et al*., 2005; Norup *et al*., 2006 and Baker *et al*., 2009).

Morphological characters that define the arecoid clade and distinguish it from other subfamilies of the family Arecaceae are the presence of reduplicate, pinnate and pinnately veined simple leaves, highly differentiated primary inflorescence bracts and flower mostly in triads (floral/ flower triad is a cluster of three unisexual flowers, comprising a central female flower flanked by two male flowers). Outside arecoids, triads flower are only found in tribe Caryoteae (Coryphoideae), which is the main reason for the erroneous placement of Caryotae within Arecoideae in the earlier classification of Uhl & Dransfield (1987). The differentiated primary inflorescence bracts of Arecoideae contrast with those of some other subfamilies, which are conspicuous and relatively uniform throughout the main axis of the inflorescence. In arecoids, however, the primary bracts subtending the first-order branches (rachis bracts) are always highly reduced, and well-developed bracts occur only on the peduncle. This feature is shared with subfamily Ceroxyloideae, the sister of Arecoideae (Asmussen & Chase, 2001; Asmussen *et al*., 2006; Baker *et al*., 2009).

The subfamily Arecoideae contains various species that are the sources of economically important palm such as oil palm (*Elaeis guineensis*), coconut (*Cocos nucifera*), betel nut palm (*Areca catechu*), peach palm (*Bactris gasipaes*) and important species in the global horticultural trade are *Dypsis lutescens* and *Roystonea regia*.

The pollen morphology of the subfamily has been studied by Erdtman 1952; Thanikaimoni1970b, 1971; Sowunmi 1972; Kedves 1980, Ferguson & Harley 1993 and Harley & Dransfield 2003. However, no detailed information on the pollen morphology of the subfamily Arecoideae is available from Pakistan and Kashmir. Therefore, pollen grains of 8 species belonging to 7 genera distributed in 3 tribes (Table 1) have been studied by using Light Microscope (LM) and Scanning Electron Microscope (SEM) from the study area.

Materials and Methods

For the study of pollen grain mature, healthy, un-dehisced and fresh flowers were collected from the field and fixed in the 70% alcohol contained in vials, whereas in few cases herbarium specimens were also taken from the herbarium of Centre for Plant Conservation University of Karachi (KUH). The pollen primarily prepared by the acetolysis method described by Erdtman (1969).

For Light Microscopy pollen were mounted in glycerine jelly stained with 1% safranine. The different

pollen characters such as length (P), breadth (E), size of colpus, exine thickness, aperture number and type was examined by using Nikon type-2 microscope under (E40, 0.65) with 10 x eye piece. The pollen data were analyzed statically i.e. calculated range, mean and standard error (±) by using MS Excel (Table 3).

For SEM, the sample was suspended in a drop of water and directly mounted on metallic stub using double sided adhesive tape. The stub was left for few hours to evaporate the water. Then the samples were coated with gold using Jeol JFC 1100 E ion sputtering device. SEM observation and photographs were carried out on a Jeol Microscope JSM6380LV.

The terminology used is in accordance to Erdtman (1952 & 1969), Faegri & Iverson (1964), Walker & Doyle (1975) and Punt *et al*. (2007). Detail of voucher specimens of the examined species is given in Table 2.

Cluster analysis: Agglomerative cluster analysis was performed choosing the Euclidean distance as the resemblance function and Ward's method for a group linkage method (McCune & Grace, 2002) so as to expose the group structure in the species on the basis of different pollen characters. The computations were performed using the computer program PC-ORD (version 6.0) (McCune & Grace, 2002; Peck, 2010). The various pollen characters of the studied taxa belonging to sub family Arecoiadeae (family Arecaceae) have been used in data matrix. The characters and character state used for performing hierarchical clustering are listed in Table 4.

Observations and Results

The summary including quantitative and qualitative pollen morphological data of the studied taxa belonging to the subfamily Arecoideae is given in the Table 3.

General Pollen Characters of the Subfamily Arecoideae

Arecoideae is a eurypalynous subfamily that shows the considerable pollen diversity within the family such as size, shape, aperture and exine pattern. The predominant aperture type is monosulcate (i.e., 7 out of 8 species are monosulcate) whereas trichotomosulcate pollen is only found in *Elaeis guineensis*. The average size of pollen is from 13–36 µm in length and 20–65 µm in breadth. The shape of the pollen grain, as seen in polar view, is frequently elliptical either symmetrical or asymmetrical but sometimes rounded to triangular pollen have also been found. On the other hand, in equatorial view the pollen is kidney- shaped in transverse position and more or less boat shaped in longitudinal position. Infratectum collumelate. Exine reticulate, perforate- rugulate, punctate, vermiculate, and spinulose but the most common pattern found is reticulate type. In most of the species, ornamentation is mottled, perforate or granulate, finely reticulate-granulate. The exine thickness ranges from 1.0–3.0 µm.

On the basis of pollen aperture, size and exine pattern, four pollen types have been recognized viz., *Areca*-type, *Dypsis*- type, *Cocos*- type and *Elaies*-type.

Table 1. List of the examined taxa of the subfamily Arecoideae (Family Arecaceae).

Subfamily	Tribe	Subtribe	Genera	Species
Arecoideae Griff.	Areceae Drude	Arecinae Hook.	*Areca* L.	*A. catechu* L.
		Dypsidinae Becc.	*Dypis* Noronha ex Mart.	*D. lutescens* (H. Wendl.) Drans. & Beentje
		Ptychospermatinae Hook.	*Ptychosperma* Labill.	*P. elegans* (R.Br.) Blume
				P. macarthuri (H. Wendl.) H. Wendl. ex Hook. f.
			Wodyetia Irvine	*W. bifurcata* Irvine
	Roystoneae Cook		*Roystonea* Cook	*R. regia* Cook
	Cocoeae Mart.	AttaleinaeDrude	*Cocos* L.	*C. nucifera* L.
		Elaeidinae Hook.	*Elaeis* Jacq.	*E. guineensis* Jacq.

Table 2. Detail of the voucher specimens of studied taxa (subfamily Arecoideae).

Name of taxon	Voucher specimens
Areca catechu	*Shagufta* s.n.(KUH); *Kamal Akhtaer* 666, 780(KUH); *Kamal Akhtar s.n.* (KUH)
Dypis lutescens	*Abid A. Rashid* 456 (KUH); *Abid A. Rashid* 459 (KUH), *Roohi Abid* 50 (KUH)
Ptychosperma elegans	*Saood Omer* 623 (KUH); *Saood Omer* 645 (KUH); *Abid A. Rashid* 448, 449 (KUH)
Ptychosperma macarthuri	*Rubina Saleem* 356 (KUH); *Saood Omer* 321 (KUH); *Roohi Abid* 40, 42 (KUH)
Wodyetia bifurcata	*Abid A. Rasheed* 357 (KUH); *Abid A. Rasheed* 387 (KUH); *Roohi Abid* 45 (KUH); *Shaukat Ali* 550 (KUH)
Roystonea regia	*Abid A. Rashid 16* (KUH); *Abid A. Rashid & Adil* 89, 33 (KUH)
Cocos nucifera	*A.A. Qureshi* s.n. (KUH); *Abid A. Rashid* 17 (KUH); *Abid A. Rashid* 19 (KUH)
Elaeis guineensis	*Abid A. Rashid* 35(KUH); *Abid A. Rashid* 40 (KUH); *Shaukat Ali* s.n. (KUH)

Table 3. General pollen characters of the subfamily Arecoideae.

Name of taxa	Tribe	Length (µm)	Breadth (µm)	Colpus length (µm)	Aperture	Exine hickness (µm)	Tectum
Areca catechu	Areceae	21.20 (23.85) 26.50 ± 0.595	37.50 (41.35) 45.20 ± 0.876	20.50 (23.50) 26.50 ± 0.639	Monosulcate	1.3 - 2	Finely reticulate
Dypsis lutescens	Areceae	15.50 (21.0) 26.50 ± 1.220	30.80 (36.70) 42.60 ± 1.25	15.50 (20.75) 26.00 ± 1.170	Monosulcate	1.5 - 2.5	Spinulose or perforate
Ptychosperma elegans	Areceae	23.20 (25.85) 28.50 ± 0.593	35.60 (39.90) 44.20 ± 0.830	21.00 (23.70) 26.40 ± 0.650	Monosulcate	1.2 - 2	Reticulate or finely perforate-rugulate
P. macarthuri	Areceae	25.20 (28.35) 31.50 ± 0.721	39.40 (41.47) 43.55 ± 0.521	25.00 (27.85) 30.70 ± 0.592	Monosulcate	1.2 - 2	Reticulate or finely perforate-rugulate
Wodyetia bifurcata	Areceae	24.60(28.50) 32.40 ± 0.784	31.50 (35.85) 40.20 ± 1.083	24.50 (28.25) 32.00 ± 0.967	Monosulcate	1.5 - 2	Reticulate or finely perforate-rugulate
Cocos nucifera	Cocoeae	23.20 (26.80) 30.40 ± 0.842	44.20 (52.15) 60.10 ± 1.734	23.20 (26.50) 29.80 ± 0.722	Monosulcate	1.5	Punctate
Elaeis guineensis	Cocoeae	31.60 (34.30) 37.0 ± 0.632	16.64 (21.16) 25.68 ± 0.907	25.20 (29.90) 34.60 ± 0.834	Trichotomosulcate	1.1	Reticulate -vermiculate
Roystonea regia	Roystoneae	40.30 (46.25) 52.20 ± 1.284	39.15 (44.20) 49.25 ± 1.002	39.50 (45.45) 51.40 ± 1.269	Monosulcate	1.5	Punctate to vermiculate

Table 4. Data matrix of the studied taxa representing subfamily Arecoideae scored for 21 characters

Name of taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Areca catechu	23.85	41.35	1.65	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0
Dypsislutescence	21.0	36.70	2	1	1	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	1
Ptychospermaelegans	23.85	41.35	1.6	1	1	0	0	1	0	0	0	1	1	0	0	1	1	0	0	1	0
P. macarthuri	33.85	41.47	1.6	1	1	0	0	1	0	0	0	1	1	0	0	1	1	0	0	1	0
Wodyetiabifurcata	23.85	41.35	1.75	1	1	0	0	1	0	0	0	1	1	0	0	1	1	0	0	1	0
Cocusnucifera	26.80	54.60	1.5	1	1	0	0	1	0	0	0	1	1	0	0	0	0	1	0	0	0
Elaeisguineensis	34.30	21.16	1.1	3	1	0	0	1	0	0	0	1	1	0	0	1	0	0	1	0	0
Roystonearegia	46.25	44.20	1.5	1	1	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0

Key to the List of characters, scored for cluster analysis for the studied taxa of the subfamily Arecoideae

Character description: 1. Length (µm)**;** 2. Breadth (µm)**;** 3.Exine Thickness (µm); 4.Aperture number; **Aperture type:** 5. Sulcate: Absent (0), Present (1)**;** 6.Porate: Absent (0), Present (1); 7. Zonosulcate: Absent (0), Present (1); **Aperture Position:** 8. Polar: Absent (0), Present (1); 9 Equatorial: Absent (0), Present (1); 10. Merdonial: Absent (0), Present (1); **Symmetry:** 11. Symmetric: Absent (0), Present (1); 12. Asymmetric: Absent (0), Present (1)**; Tectum:** 13.Tectate including semitectate: Absent (0), Present (1); 14. Intectate: Absent (0), Present (1); 15. Supratectal process: Absent (0), Present (1); **Exine pattern:** 16. Reticulate (including sub-reticulate)**:** Absent (0), Present (1)**;** 17. Perforate**;** 18.Punctate: Absent (0), Present (1)**;** 19. Vermiculate:Absent (0), Present (1);20. Rugulate:Absent (0), Present (1); 21. Spinulose:Absent (0), Present (1)

Key to the pollen types

1 + Pollen trichotomosulcate ..... *Elaeis* type (*E. guineensis*)
- Pollen monosulcate ……..……..……..……..……..... 2
2 + Pollen more than 45 µm in length. Tectum punctate to vermiculate ……..……... *Cocos* type (*C. nucifera* and *R. regia*)
- Pollen up to 45 µm in length. Tectum not as above ……..……..……..……..…… 4
3 + Pollen spinulose, spinules borne on the tectum ……..……..……..….. *Dypsis* type (*D. lutescens*)
- Tectum finely reticulate or finely perforate-rugulate ……..……..... *Areca* type (*A. catechu*, *P. excelsa, P. macarthuri* and *W. bifurcata*)

Pollen description of the tribe Areceae

Pollen monosulcate, elliptical or circular, asymmetric. Size: 15- 32 µm in length and 30-45 µm in breadth. Colpus length 14-32 µm. Tectum reticulate, perforate or finely perforate-rugulate or spinulose, spinules borne on the tectum. Muri simpli-through to multi baculate.

The tribe is represented by 4 genera viz., *Areca* L., *Dypsis* Noronha ex Mart., *Ptychosperma* Labill. and *Wodeytia* Irvine (Table 3).

Key to the genera

1 + Tectum finely perforate-rugulate or reticulate. Aperture equal in length to the longer axis of pollen …… ……..……..……..... ***Ptychosperma group* (3. *Ptychosperma* & 4.*Wodeytia)***
- Tectum not as above. Aperture shorter than or as long as the length of the longer axis of pollen …….. ……..……..……..…….. **2**
2 + Tectum finely reticulate. Muri simple baculate to dupli-baculate ……..….…..... **1. *Areca***
- Tectum spinulose. Muri not as above……..……..……..... **2. *Dypsis***

1. *Areca* L.

Pollen monosulcate, elliptic in polar view; boat-like in equatorial view, exine finely reticulate.

Areca catechu L. (Fig. 1.1 A & B; 2A)

Pollen (21.20-) 23.85 (-26.50) µm in length and (37.50-) 41.35 (-45.20) µm in breadth, asymmetric. Colpus length (23.50-) 24.00 (-26.50) µm, margin wavy. The aperture is equal or shorter than the longest axis of pollen. Exine tectate or semitectate, 1.3–2.0 µm thick. Tectum 0.8 µm thick, finely reticulate. The muri is 0.6 µm wide, simple baculate.

2. *Dypsis* Noronha ex Mart.

Pollen monosulcate, elliptic to pyriform in polar view; boat-like in equatorial view. Tectum spinulose.

Dypsis lutescens (H.Wendl.) Dransf. &Beentje (Fig. 1.1 C & D; 2B)

Pollen (15.50-) 21.00 (-26.50) µm in length and (30.80) -36.70 (-42.60) µm in breadth, usually asymmetric. Colpus length (15.50-) 20.75 (-26.00) µm. The aperture is shorter than, or as long as, the longest axis of pollen. Exine is usually tectate occasionally semitectate, 1.5-2.5 µm thick. Tectum spinulose. Muri at reticulum coarsely granulate spinuous.

3. *Ptychosperma* Labill.

Pollen monocolpate, ellipsoidal in polar view occasionally pyriform; boat-like in equatorial view. Tectum finely perforate-rugulate or reticulate. Exine tectate, 1.2-2.0 µm thick. Aperture margin similar or slightly finer.

Ptychosperma elegans (R.Br.) Blume (Fig. 1.1 E & F; 2C)

Pollen (22.40-) 24.95 (-27.50) µm in length and (31.60-) 36.40 (-41.20) µm in breadth, asymmetric. Colpus length (21.20-) 24.10 (-27.00) µm. The aperture is about equal in length to the longer axis of pollen.

Ptychosperma macarthuri (H. Wendl.) H. Wendl. ex Hook. f. (Fig. 1.1 G & H; 2D)

Pollen (25.20-) 28.35 (-31.50) µm in length and (39.40-) 41.47 (-43.55) µm in breadth, asymmetric Colpus length (25.00-) 27.85 (-30.70) µm. The aperture is about equal in length to the longer axis of pollen.

4. *Wodeytia* Irvine

Pollen monosulcate, elliptic in polar view; boat-like in equatorial view. Tectum reticulate or finely perforate-rugulate.

Wodeytia bifurcata Irvine (Fig. 1.2 A & B; 2E)

Pollen (24.60-) 28.50 (-32.40) µm in length and (31.50-) 35.85 (-42.20) µm in breadth, slightly asymmetric. Colpus length (24.50-) 28.25 (-32.00) µm. The aperture almost equalling the longer axis of pollen. Exine tectate, 1.5-2.0 µm thick.

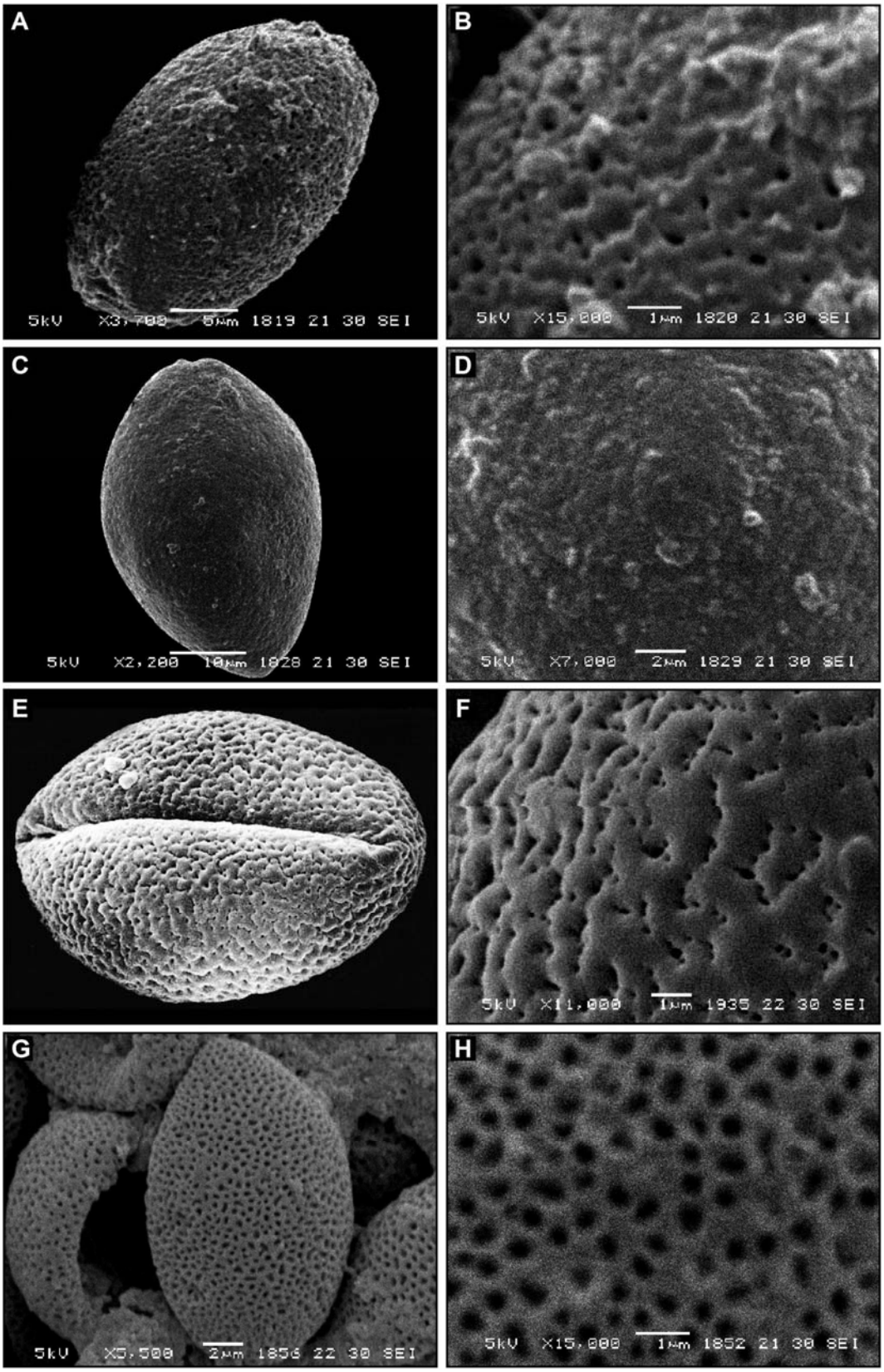

Fig. 1.1. Scanning Electron Micrographs (SEM) of the pollen: *Areca catechu*: A, pollen grain; B, exine pattern. *Dypsis lutescens*: C, pollen grain; D, exine pattern. *Ptychosperma elegans*: E, pollen grain; F, exine pattern. *Ptychosperma macarthuri:* G, pollen grain; H, exine pattern (scale bar: B, F, H = 1µm; D, G = 2; A= 5µm; C = 10 µm).

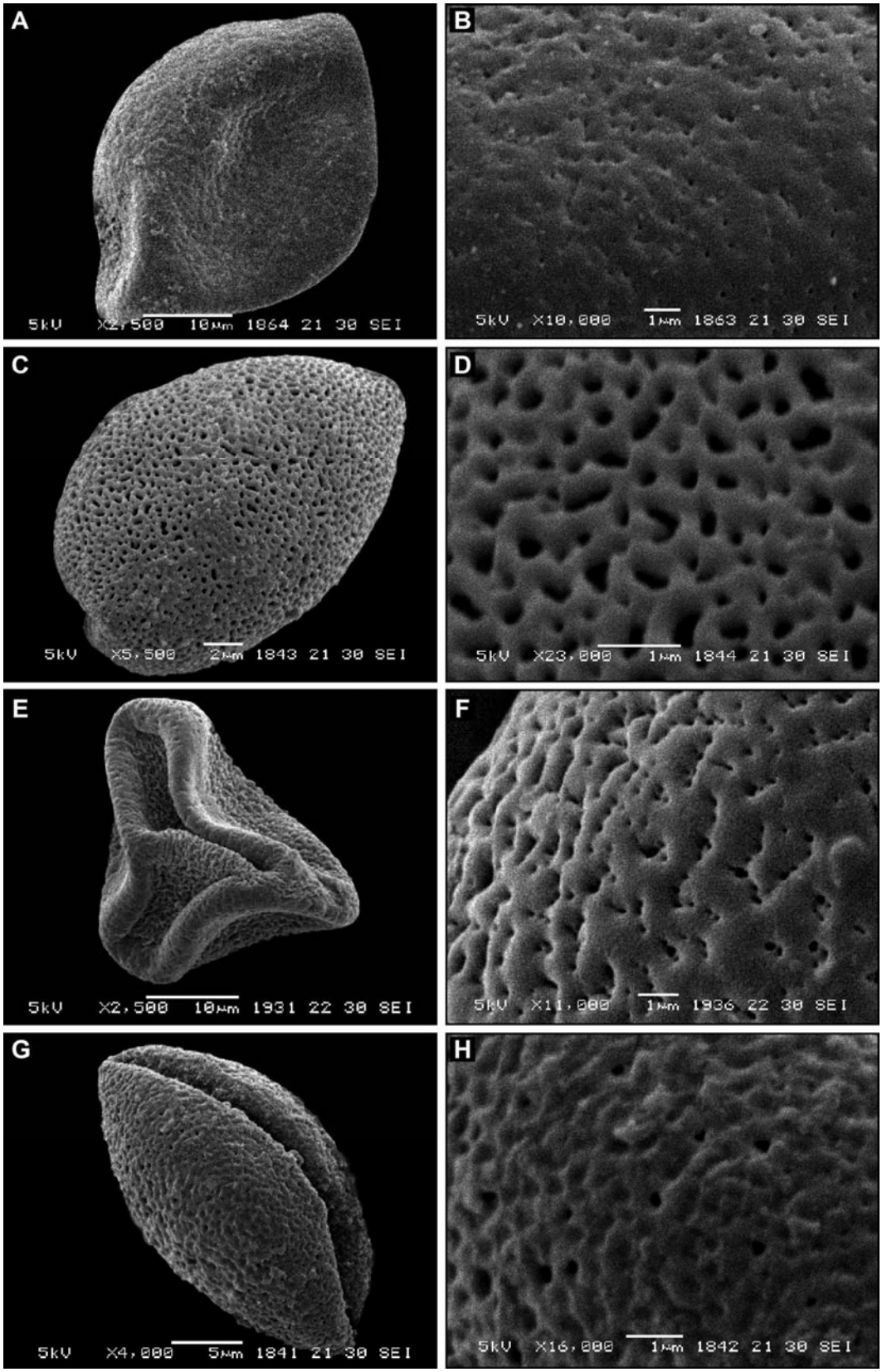

Fig. 1.2. Scanning Electron Micrographs (SEM) of the pollen: *Wodeytia bifurcata*: A, polar view; B, exine pattern. *Cocus nucifera*: C, polar view; D, exine pattern**.** *Elaeis guineensis***:** *E, polar view*; F, exine pattern. *Roystonea regia:* G, polar view; H, exine pattern (scale bar: B, D, F, H = 1µm; C = 2 µm; A, G = 5 µm; E = 10 µm).

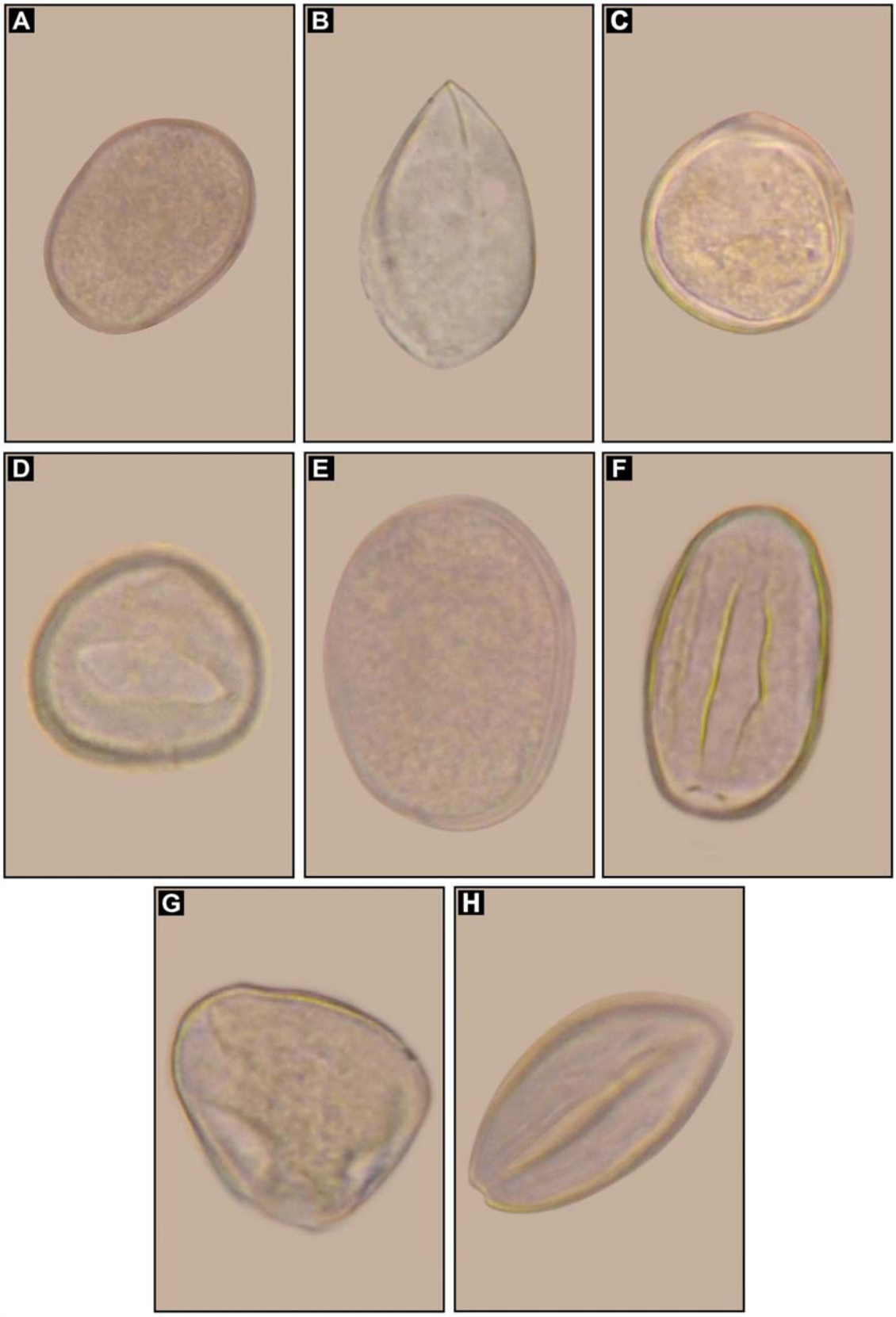

Fig. 2.3 Light Micrographs (LM) of the pollen grains at 100 x: A, *Areca catechu*: B, *Dypsis lutescens*: C, *Ptychosperma elegans*: D, *Ptychosperma elegans*: E, *Wodyetia bifurcata*; F, *Cocus nucifera*: G, *Elaeis geneissis*: H, *Roystonea regia*: (Scale Bar = 10µm)

Pollen description of the tribe Cocoeae

Pollen elliptical to more less circular or oblate-triangular in apertural view, usually asymmetric. Size: (23.20-) 26.80 (-30.40) µm in length and (44.20-) 54.60 (-65.00) µm in breadth. Aperture monosulcate or trichotomosulcate. Colpus 18-65 µm in length, margin wavy or broad and psilate. Exine tectate or semi tectate, up to 2.0 µm thick. Tectum 0.6 µm thick, punctate - perforate or reticulate to vermiculate.

The tribe comprises of 2 genera viz., *Cocos* L. and *Elaeis* Jacq. (Table 3)

Key to the genera

1 + Pollen monosulcate, up to 65 µm long ……..…..……..……..... **1. *Cocos***
\- Pollen trichotomosulcate, up to 37 µm long ……..……..…..…… **2. *Elaeis***

1. *Cocos* L.

Pollen monosulcate, elliptical to more or less circular in polar view. Tectum punctate.

Cocos nucifera L. (Fig. 1.2 C & D; 2F)

Pollen (24.60-) 28.50 (-32.40) µm in length and (44.20-) 52.15 (-60.10) µm in breadth, frequently elongate, usually asymmetric. Colpus (23.20-) 26.50 (-29.80) µm in length. Exinetectate or semi tectate, 1.5 µm thick, undulating, segments with exinous enfolding tubercles. Tectum 0.6 µm thick, perforation punctate supported by a finely but densely baculate layer.

2. *Elaeis* Jacq.

Pollen trichotomosulcate, triangular in polar view. Tectum reticulate to vermiculate.

Elaeis guineensis Jacq. (Fig. 1.2 E & F; 2G)

Pollen (31.60-) 34.30 (-37.00) µm in length and (16.64-) 21.16 (-25.68) µm in breadth, radio-symmetrical with concave sides. Colpus (31.60-) 34.30 (-37.00) µm in length. Aperture margin wavy, broad and psilate, not always reaching the periphery of the pollen. Exine tectate or semitectate, 1.1 µm thick. Tectum 0.6 µm thick. Bacula present.

Pollen description of the tribe Roystoneae

1. *Roystonea* O. F. Cook

Pollen monosulcate, elliptic in polar view; boat-like in equatorial view. Tectum punctate to vermiculate.

Roystonea regia (H. B.&K.) O.F. Cook (Fig. 1.2 G&H; 2H)

Pollen (40.30-) 46.25 (-52.20) µm in length and (39.15.-) 44.20 (-49.25) µm in breadth, with slight or obvious asymmetric.Colpus length (39.50-) 45.45 (-51.40) µm. The aperture almost equals to the longer axis of pollen. Exine tectate, 1.5 µm thick.

Discussion

The subfamily Arecoideae is probably the most varied, including morphologically (Moore, 1960) and palynological variation (Erdtman, 1952; Sowunmi, 1972), but uniform anatomically (Tomlinson, 1961).The pollen of the subfamily Arecoideae were found to be diverse with regard to aperture forms such as monocolpate, monoporate, annuporate, trichotomocolpate, and triporate (Sowunmi, 1972). Although there are many genera with the generalized monosulcate type (Harley, 1990) but sometimes associated with trichotomosulcate pollen as recorded in the literature (Harley, 1999).The present results also show that out of 8 taxa, 7 have monosulcate pollen while *Elaeis* Jacq. is represented by trichotomosulcate pollen. Some earlier workers such as Thanikaimoni, 1970; Sowunmi, 1972 and Ferguson 1986 have found trichotomosulcate pollen in some others Arecoid also, such as *Pinanga, Bactris, Astrocaryum,* and *Acrocomia*. Similarly, the cluster analysis of the 8 OTUs (subfamily Arecoideae) based on pollen morphology show two distinct groups (Fig. 3) where as on the basis of the exine pattern and aperture of pollen four types have been recognized in the sub family Arecoideae. These pollen types are *Elaeis* type (*E. guineensis*), *Cocos* type (*C. nucifera* and *R. regia*), *Dypsis* type (*D. lutescens*), *Areca* type (*A. catechu*, *P.excelsa, P. macarthuri* and *W. bifurcata*).

Tribe Areceae

In this tribe, pollen morphology of the 5 taxa belonging to 3 subtribes viz., Arecinae (*Areca catechu*), Dypsidinae (*Dypsis lutescens*) and Ptychospermatinae (*Ptychosperma elegans, Ptychosperma macarthuri* and *Wodyetia bifurcata*)) were studied, which falls in to 2 pollen types on the basis of exine features viz., *Areca*-type and *Dypsis*-type. However, monosulcate pollen is present throughout the tribe. As far as the cladistic analysisis concerned, the group-1 represents all the studied species of the tribe Areceae. Among these, *D. lutescens* does not form cluster to any species of the Arecoideae due to the difference in their pollen size and exine pattern but partially linked as it share same pollen aperture (Tables 3 & 4; Fig. 3). From the members of *Areca*–type, *W. bifurcata* appears to be closely linked with *A. catechu* as both the taxa have same pollen size and delimited on the exine pattern, whereas both the species of *Ptychosperma* falls in between these two taxa. The differences are found in their pollen size and also in the exine pattern. For instance, in *Areca* exine is finely reticulate while in *Ptychosperma* and *Wodeytia* exine is reticulate or perforate to rugulate (Tables 3 & 4; Fig, 1.1 A, B & E – H; Fig. 1.2 A, B). Similarly, the following genera viz., *Ptychosperma* and *Wodyetia* show close association with each other due to presence of same pollen characters Likewise, in terms of gross morphological characters both these genera are closely related, as indicated by their inclusion of same subtribe Ptychospermatinae and only classified on the basis of seed characters such as seeds terete in *Wodyetia* and angled in *Ptychosperma.* Some previous workers such as Thanikamoni (1971), Sowunmi (1968, 1972), Kedves (1980), (Dransfield & Uhl, 1998) and Dransfield *et al*. (2005, 2008) also observed the almost same characters within the tribe Areceae.

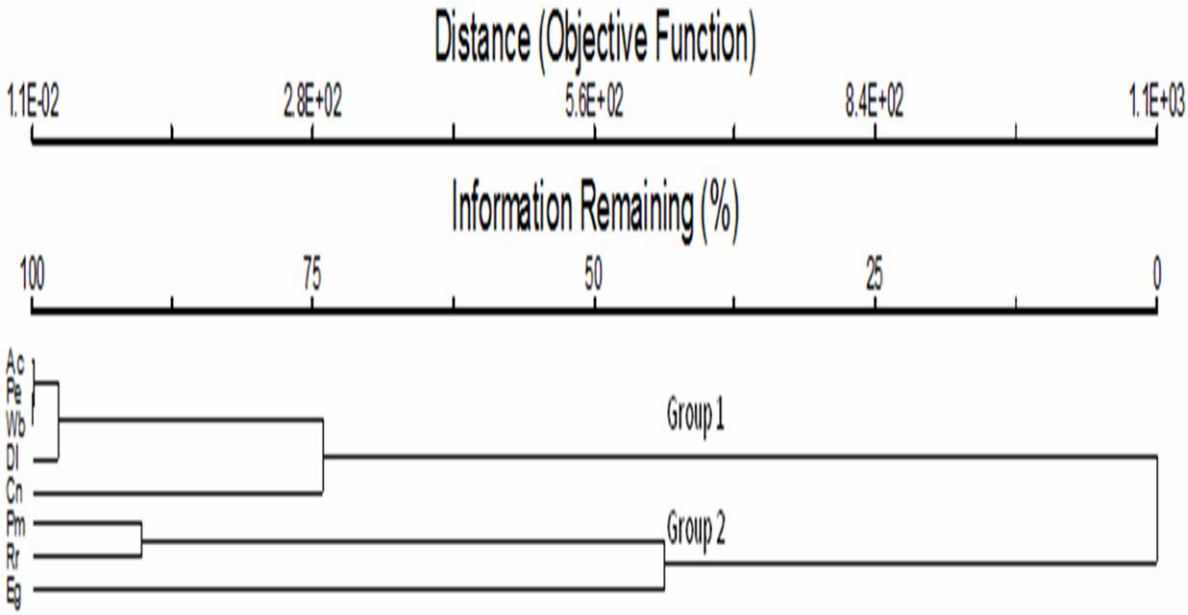

Fig. 3. Dendrogram obtained by Ward's cluster analysis, showing two groups of species belong to family Arecoiadeae, separated on the basis of different pollen characteristics.

Key to abbreviations: Ac = *Areca catechu,* Dl = *Dypsis lutescens,* Pe = *Ptychosperma elegans,* Pm = *Ptychosperma macarthuri,* Wb = *Wodyetia bifurcata,* Cn = *Cocos nucifera,* Eg = *Elaeis guineensis,* Rr = *Roystonea regia.*

Tribe Cocoeae

This tribe is represented by the two genera i.e., *Cocos* (*C. nucifera*) and *Elaeis* (*E. guineensis*). These genera can be easily distinguished on the basis of aperture and exine pattern. In the former genus, the pollen is monosulcate and punctate whereas, in the later genus, the pollen is trichotomosulcateand reticulate-vermiculate (Tables 3 & 4; Fig, 1.2 C- F). The cluster analysis also clearly indicates that *E. guineensis* occupy basal position and do not form any clade with other arecoids as the genus is characterized by trichotomosulcate pollen (Fig. 3). Some previous workers such as Mahabalé (1967), Thanikamoni (1971), Sowunmi (1968, 1972), Kedves (1980), Harley (1990) and Harley & Dransfield (2003) also treated these genera in to two different groups. It is also noted that between these genera there is an extraordinary distinction in morphological characters. The genus *Elaeis* is distinguished by the presence of unarmed petiole; bisexual inflorescence; beaked fruit and endocarp with apical pore. On the other hand, *Cocos* is characterized by having armed petiole, unisexual inflorescence, unbeaked fruit and endocarp with basal pore. Thus these genera fall into two subtribes viz., Attaleinae (*Cocos*) and Elaeidinae (*Elaeis*) shows that both the genera appear to be closely related as they fall in the same tribe but definitely distinct.

Tribe Roystoneae

This tribe is represented by a single species viz., *R. regia*. This species is characterized by punctate to vermiculate tectum. Thanikaimoni (1970 b) and Sowunmi (1972) also described the pollen as monosulcate, elliptical in polar view, tectum punctate to vermiculate. Cladistically, the genus *R. regia* falls in group-2 and show a close linkage with *C. nucifera* (Fig. 3) as they have monosulcate pollen and punctate tectum. Therefore, this taxon included in the following pollen type (viz., *Cocos*-type).

Although, the present investigations clearly indicate that pollen morphology of the family Arecaceae fully support the delimitation at generic level or even at the higher level also. Therefore various workers such as Mahabale (1967), Thanikaimoni (1971), Sowunmi (1968, 1972), Kedves (1980), Ferguson (1986), Ferguson & Harley (1993), Dransfield *et al*. (1998, 2008) separated arecoid palms mainly on the basis of pollen shape, aperture type and exine ornamentation. However, at the specific level pollen data are not helpful because species of the representative genus have uniform pollen. The cluster analysis of the subfamily Arecoideae also support the grouping based on the pollen morphological data.

It is interesting to note that in some cases, pollen morphology shows a remarkable correlation with gross morphology (as in the subtribe Ptychospermatinae (*Ptychosperma* and *Wodyetia*)) on the other hand they also show a lack of correlation as in the tribe Cocoeae (*Cocos* L. and *Elaeis* Jacq.).

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