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POLLEN MORPHOLOGY OF THE SUBFAMILY CORYPHOIDEAE –ARECACEAE (EXCLUDING TRIBE PHOENICEAE) FROM PAKISTAN AND KASHMIR

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Abstract

The pollen morphological diversity of the family Arecaceae has been widely demonstrated, and foregoing studies have revealed that pollen characters may contribute to a better understanding of the systematics of taxa. The present study was carried out to examine the pollen morphological characters of 14 species representing 12 genera of the subfamily Coryphoideae (excluding the tribe Phoeniceae) by using Light microscope (LM) and Scanning electron Microscope (SEM). In general, the pollen throughout the subfamily is monosulcate and elliptic in apertural view. However, the differences are found in the pollen size (ranges from 13.50 µm to 51.00 µm), exine thickness (i.e., from 1.0 µm to 3.0 µm), exine pattern (either supra tectal gemmae present or absent) and the number of baculae (i.e., one, two or more). Pollen characters have been found to be taxonomically useful for characterization of the genera. Therefore, on the basis of the combination of pollen morphological characters four pollen types have been recognized such as *Bismarkia*-type, *Borassus*-type, *Brahea*-type and *Sabal* type. The pollen morphological data (quantitative and qualitative) have also been analyzed by Agglomerative cluster analysis choosing the Euclidean distance and Ward's method for a group linkage method.

Key words: Coryphoideae, Arecaceae, Pakistan.

Introduction

The subfamily Coryphoideae is monophyletic and resolves in most phylogenetic studies as sister to a clade including the Arecoideae and the Ceroxyloideae (Asmussen *et al*., 2006; Dransfield *et al*., 2008; Baker *et al*., 2009). A subfamily with about 44 genera and c. 450 species belonging to 8 tribes, distributed globally, with centre of diversity in the New World and Asia/Malaysia (Dransfield *et al*., 2008). The subfamily Coryphoideae can be easily discerned by the presence of combination of characters such as leaves palmate or costapalmate, induplicate, rarely reduplicate (when flowers is apocarpous), or mixed induplicate-reduplicate, or pinnate. Flower solitary or clustered; never arranged in triads of one central pistillate flower and two lateral staminate flowers. The combinations of these characters segregate the Coryphoideae from other subfamilies. An indication of the importance of Palms in ancient time is that they are mentioned more than 30 times in Bible and at least 22 times in the Quran (Date palm (*Phoenix dactylifera* L., is the oldest cultivated tree crop). Moreover, number of palms are economically important such as leaves and other parts of *Nannorrhops ritichiana*, *Brahea brandegeei* and *Livistonia chinensis* are used for thatching or weaving and for fuel. Similarly, various coryphoid palms such as *Livistonia chinensis* (China palm), *Caryota urens* (Wine palm or Fish tail palm), *Sabal minor* (Bush palmetto) are universally known for their majestic look and elegant shapes, and have commercial horticultural importance too.

Palms are highly distinctive at the family level but within the family their morphological diversity is probably greater than that of any other monocotyledonous family (Uhl & Dransfield, 1987).At the level of pollen morphology, the same diversity is also found such as shape and size of pollen, ornamentation and ultrastructure of exine, form, number and arrangement of pollen aperture, all show a wide range of variation. Due to the pollen diversity, the extensive work has been carried out on the palynological studies of the family Palmae (including subfamily Coryphoideae) by various workers such as Mahabalé (1967), Thanikaimoni (1966, 1970a, b), Sowunmi (1968), Kedves (1981), Ferguson (1986), Ferguson *et al*. (1993, 1987), Dransfield *et al*. (1990), Harley (1990, 1999), Ferguson & Harley (1993), Harley & Baker (2001) and Harley & Dransfield (2003). Thanikaimoni (1970a), Sowunmi (1972) comprehensively described and illustrated the pollen morphology of the tribe Coryphoideae using Light microscope. Ferguson *et al.* (1987) provided a detailed account on the pollen morphology of the tribe Borasseae with the help of Scanning Electron Microscope and proposed that the pollen characters could be useful in the taxonomy of the tribe. Dransfield *et al*. (1990) gave an account on the pollen of the Coryphoid palms including the SEM microphotographs. Ferguson & Harley (1993) examined the pollen of the tribe Coryphoideae in detail with electron microscope and also discussed the taxonomic significance of pollen morphology.

Although, from our region, few studies have been conducted to date. For instance, Rashid & Perveen (2014) carried out the palynological studies of the tribe Phoeniceae (Coryphoideae-Arecaceae) and resolved that the delimitation of species based on pollen characters is difficult within the entire tribe but in combination with other morphological characters the species can be easily delimited. Rashid *et al*. (2016) studied the pollen morphology of 8 taxa of the subfamily Arecoideae (Arecaceae) by using LM and SEM and concluded that species representing the Arecoid palms fully support the delimitation of generic level or even at the higher level whereas at the specific level pollen data are not helpful because species of the representative genus have uniform pollen.

The primary aim of the present work is to study the pollen morphology of the subfamily Coryphoideae (excluding Phoeniceae) from Pakistan and Kashmir and in order to assess its importance in classification and delimitation of studied taxa at species or even at higher level using LM and SEM. These palynological characters have been numerically analyzed in order to quantify the phylogeny of the coryphoid palms.

Materials and Methods

For the study of pollen grains, 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 polleniferous material was also taken from the herbarium specimens of Centre for Plant Conservation, University of Karachi (KUH). The pollen material was primarily prepared by the acetolysis method, described by Erdtman (1969).

For Light Microscopy (LM) pollen were mounted in glycerine jelly stained with 1% safranine. The following pollen characters such as length (P), breadth (E), size of colpus, exine thickness and ornamentation aperture number and type (Table 3), was examined by using Nikon type-2 microscope under (E40, 0.65) with 10 x eye piece. The pollen data were analyzed statistically i.e. calculated range, mean and standard error (±) by using MS Excel (Table 3). The measurements are based on 10-15 readings from each specimen.

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) and Punt *et al*. (2007). A list of studied species of the coryphoid palms along with their localities is given in Table 2.

Cluster Analysis:

Agglomerative cluster analysis was made by 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 studied taxa on the basis of different pollen morphological characters. The computations were performed using the computer program PC-ORD (version 6.0) (McCune & Grace, 2002 and Peck, 2010).The various pollen characters of the studied taxa belonging to subfamily Coryphoideae (family Arecaceae) have been used in data matrix. The characters and character state used for performing hierarchical clustering are listed in tables 4 and 5.

Observations and Results

The summary of quantitative and qualitative pollen morphological data of the studied taxa belonging to the sub-family Coryphoideae is given in the Table 3. The SEM photographs are given in Figs. 1-3.

General Pollen Characters of the Subfamily Coryphoideae

Pollen monosulcate, usually elliptical or circular to subcircular in apertural view. Size: (13.80-) 29.00 (-51.00) µm in length and (20.00-) 27.35 (-50.00) µm in breadth. Aperture membrane is smooth, thin and usually narrow, inconspicuous aperture margin. Aperture length is almost to the size of longest axis in polar view (i.e., 32-51 µm). Colpus 14.50 (31.25) 48.00 µm in length. Exine tectate or semitectate, 1.0–3.0 µm thick, supratectal process present or absent. Exine ornamention in general may be verrucose, rugulate, finely or densly reticulate and perforate, with supratectal gemmae but the most common pattern found is reticulate type. Muri or sexine simple baculate to dupli or tripli to multibaculate.

Pollen description of the tribe Borasseae

Pollen usually elliptical or subcircular in apertural view. Size: Pollen (34.00-) 45.00 (-51.00) µm in length and (26.00-) 36.50 (-40.00) µm in breadth. Aperture length is almost to the size of longest axis in polar view (i.e., 32 - 51 µm). Colpus 33.50 (44.80) 49.80 µm in length. Exine tectate or semitectate, 1.0–3.0 µm thick, supratectal process present or absent. The exine ornamention in general may be verrucose, rugulate or reticulate, or finely perforate with supratectal gemmae.

The tribe is represented by 3 genera viz., *Bismarkia* Hildebr. & H. Wendl, *Borassus* L. and *Hyphaene* Gaertner (Tables 1 & 3).

Table 1. Synopsis of the tribes, subtribes and genera of Coryphoideae (excluding Phoeniceae) from Pakistan and Kashmir.

Subfamily	Tribe	Subtribe	Genera	Species
Coryphoideae Griff.	Borasseae Mart.	Hyphaeninae Becc.	*Bismarkia* Hildeb & H. Wendl.	*B. nobilis*
			Hyphaene J. Gaertn.	*H. thebaica* (L.) Mart.
		Lataniinae Meis.	*Borassus* L.	*B. flabellifera* L.
	Caryoteae Drude		*Caryota* L.	*C. urens*L.
	Corypheae Mart.	Coryphinae Drans. & Uhl	*Nannorrhops* H. Wendl.	*N. ritichiana* (Griff.) H. Wendl.
	Trachycarpeae Drans., *et al*.,	Livistoninae Saakov	*Brahea* Mart. ex Endl.	*B. brandegeei* (Purpus) Moore
			Livistonia R. Brown	*L. chinensis* (Jacq.) R.Br. ex Mart.
			Pritchardia Seemann & H. Wendl	*P. beccariana* Rock
			Washingtonia H. Wendl.	*W. filifera* (Linden) H. Wendl.
				W. robusta H. Wendl.
		Thrinacinae Becc.	*Rhapi*s L. f. ex Aiton	*R.excelsa* (Thunb.) Henr. ex Rehd.
				R. multifida Burret
			Trachycarpus H. Wendl.	*T. fortunie* (Hook.f.) H. Wendl
	Sabaleae Mart.		*Sabal* Adanson	*S. minor* Adanson

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

Name of Taxon	Voucher specimens
Bismarkia nobilis	Karachi University Botanical Garden, Karachi, 10-03-2009, *Abid A. Rashid* 3 (KUH); ibid, *Abid A. Rashid* 4(KUH); KIBGE, University of Karachi, 18-5-2010, *Abid A. Rashid* 25 (KUH); Stadium road, Karachi, 17-05-2010, *Shaukat Ali* 34 (KUH).
Borassus flabellifera	P.E.C.H.S Block 2, 12-3-2009, *Abid A. Rashid* 17 (KUH); Bahria town, Rawalpindi, 14-4-2011, *Abid A. Rashid* 65 (KUH); Sowan Garden, Islamabad, 20-04-11, *Abid A. Rashid & Adil* 69 (KUH); Botanical Garden, University of Karachi, 12-08-2012, *Abid A. Rashid* s.n. (KUH).
Brahea brandegeei	Navy Housing Scheme, near Gizri road, Karachi, 4-7-2012, *Abid A. Rashid* 136 (KUH); Shaheed-e-millat road Karachi, 4-7-2012 *Abid A. Rashid* 139 (KUH).
Caryota urens	Ghandi garden Karachi, 02-10-77, *Kamal Akhtar Malik* 665 (KUH); ibid, *Kamal Akhtar Malik* 667 (KUH). North Nazimabad Sakhi Hasan Karachi, 7-5-1978, *Kamal Akhtar Malik* 844 (KUH)
Hyphaena thebaica	Karachi University Botanical Garden, Karachi, 10-1-2011; *Abid A. Rashid* 22 (KUH); Near Maskan Chowrangi Gulshan-e-Iqbal, Karachi, 13-02-2014; *Roohi Bano* 58 (KUH).
Livistonia chinensis	Peshawar on road side, 25-08-1978, *S.Nazimuddin &Sultan Abedin* 1200 (KUH); Ghandi garden Karachi, 02-10-1977, *Kamal Akhter Malik* 663 (KUH); Mazar-e- Quaid-e-Azam Karachi, 12-03-1978, *Kamal Akhter Malik* 785 (KUH); Lawarence garden Lahore, 01-07-1978, *Kamal Akhtar Malik* 1184 (KUH).
Nannorrhops ritichiana	c.25 km from Awaran, 21-9-1986, *Abdul Ghaffoor & Saood Umer* 1731 (KUH); c. 25 km from Awaran on way to Mangri Mashke, Awaran Khuzdar road, 8-3-1990, *Abdul Ghaffoor & Steve M. Goodman* 4419 (KUH); Bela Awaran road, 33 miles before Awaran, *S. I. Ali, S. A. Farooqi & Sultan Abedin* 1443 (KUH); c.40 miles from uthal on way to Khuzdar, 21-6-2009, *Abid A. Rashid & Hamid* 13 (KUH).
Rhapis excelsa	Botanic Garden Center For Plant Conservation University of Karachi, Karachi, 20-03-2010, *Abid A. Rashid* 132 (KUH); ibid, *Abid A. Rashid* 134 (KUH).
Rhapis multifida	Botanic Garden Center For Plant Conservation University of Karachi, Karachi, 20-03-2010, *Abid A. Rashid* 136 (KUH);Near Tool Plaza on way to Hyderabad, 6-2-2012, *Abid A. Rashid* 164 (KUH)
Sabal minor	Clifton Karachi, 5-1-2012, *Abid A. Rashid* 164 (KUH); Gulshan-e-Maymar on way to Dream world Karachi, 11-05-2012, *Abid A. Rashid* 168 (KUH)
Trachycarpus fortunei	Chanderigur road Karachi, 20-5-1978 *Kamal Akhter Malik* 47 (KUH); Amir Khusro road,2-6-2011, *Abid A. Rashid* 77 (KUH); Alfalah, PECHS Block- 2, 22-6-2011, *Abid A. Rashid*82 (KUH)
Washingtonia filifera	Near Feroz sons Ibrahim Chanderigur road Karachi, 20-4-1978, *Kamal Akhter Malik*, 839 (KUH); ibid; Shahra-e- Faisal, Karachi, 18-4-2011, *Abid A. Rashid* 66 (KUH); Karachi University Campus, Karachi, 5-2-2012, *Abid A. Rashid* 99 (KUH); Near Star gate on way to Karachi Airport, 9-3-2012, *Abid A.Rashid & Shaukat* 104 (KUH).
Washingtonia robusta	Abrahim Chanderigur road, T&T office Karachi 20-5-1978 *Kamal Akhter Malik*847 (KUH); SMI University, Karachi, 5-5-2013, *Roohi Bano* s.n (KUH); Near post office Shahr-e-Faisal, 22-6-2011 *Abid A. Rashid* 84 (KUH)

Key to the genera

1. + Supratectal gemmae present …..…….…….…….…….. **2**
   - Spratectal gemmae absent ..…….…….....……. **1. *Bismarkia***
2. + Pollen more than 45 µm long. Columella 0.6 µm high, dense. Tectum 0.8 µm thick ...……...… **2. *Borassus***
   - Pollen less than 45 µm long. Columella 0.3 µm high, less dense. Tectum 0.4 µm thick ...……. **3. *Hyphaene***

1. *Bismarkia* Hildebr. & H. Wendl.

Pollen elliptical to sub-circular in polar view. Tectum rugulate, supratectal gemmae absent.

Bismarkia nobilis Hildebr. & H. Wendl. (Fig. 1C & D)

Pollen (42.50-) 45.95 (-49.40) µm in length and (26.00-) 28.80 (-31.60) µm in breadth, asymmetrical. Aperture equal or somewhat shorter than the length. Colpus (41.0-) 44.80 (48.60-) µm in length. Exine tectate, 1.0 µm thick. Tectum 0.8 µm thick, rugulate or reticulate, perforate.

2. *Borassu*s L.

Pollen elliptical in polar view. Tectum reticulate-verrucose, supratectal gemmae present.

Borassus flabellifer L. (Fig. 1I)

Pollen (39.00-) 45.00 (-51.00) µm in length and (33.00-) 36.50 (-40.00) µm in breadth. Aperture long (equal or somewhat shorter than the length). Colpus (37.5-) 43.65 (-49.80) µm in length. Exine tectate, 2.0–3.0 µm thick including gemmae. Tectum 0.8 µm thick including gemmae.

3. *Hyphaene* Gaertner

Pollen elliptical to sub circular in polar view. Tectum reticulate-verrucose, supratectal gemmae present.

Hyphaene thebaica (L.) Mart. (Fig. 1G & H)

Pollen (34.00-) 36.00 (-40.00) µm in length and (31.00-) 34.30 (-38.00) µm in breadth. Aperture equal or somewhat shorter than the length. Colpus (33.50-) 36.25 (-39.00) µm in length.Exine tectate, 2.0-3.0 µm thick including gemmae. Tectum 0.4 µm thick including gemmae.

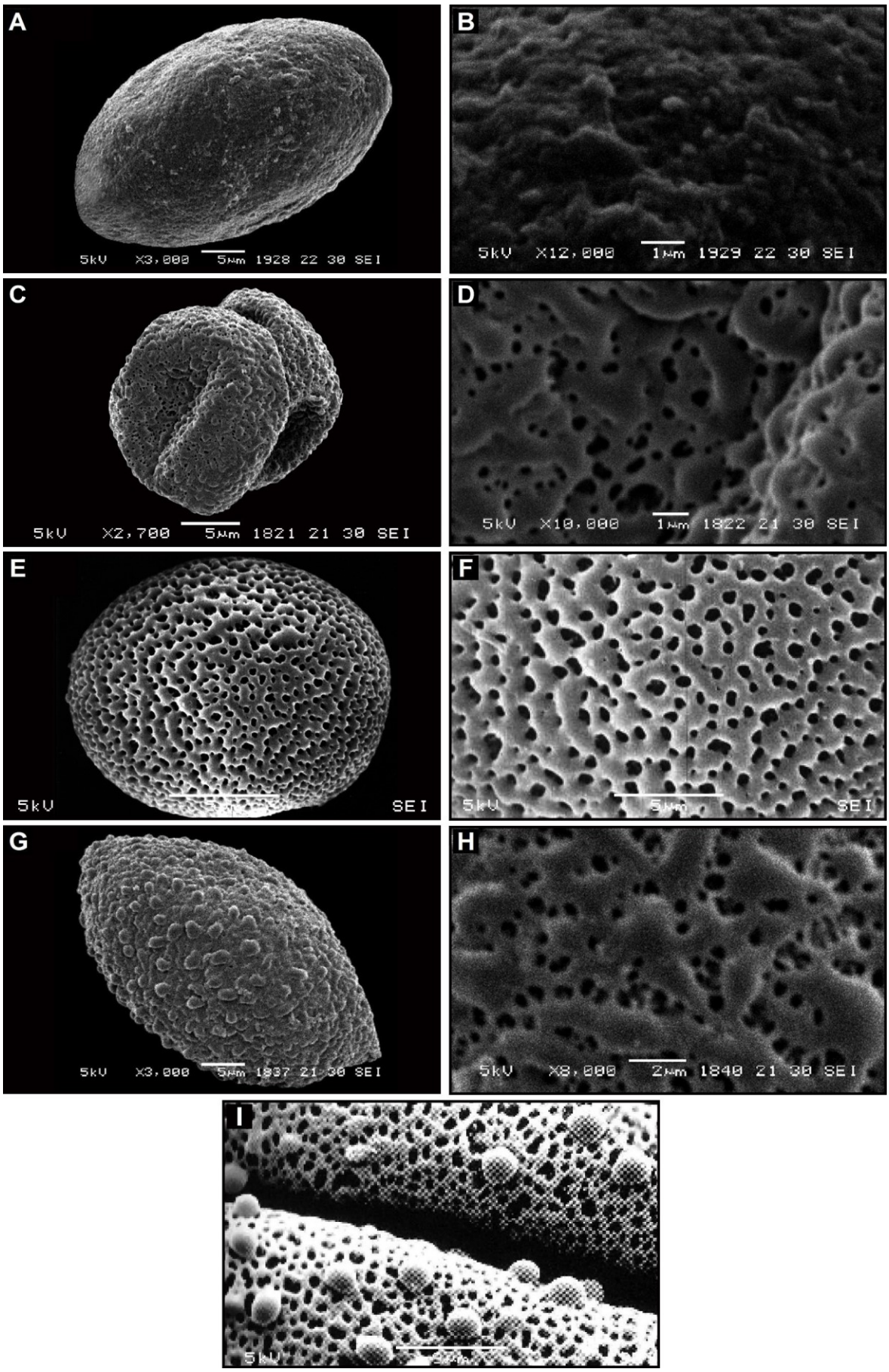

Fig. 1. Scanning Electron Micrographs (SEM): *Caryota urens*: A, pollen; B, exine pattern. *Bismarkia nobilis*: C, pollen; D, exine pattern. *Pritchardia beccariana*. E, pollen; F, exine pattern. *Hyphaene thebaica*: G, pollen; H, exine pattern. *Borassus flabellifer*: I, exine pattern (scale bar: B = 1 µm, D, H = 2 µm; A, C, F, G = 5 µm; E = 10 µm).

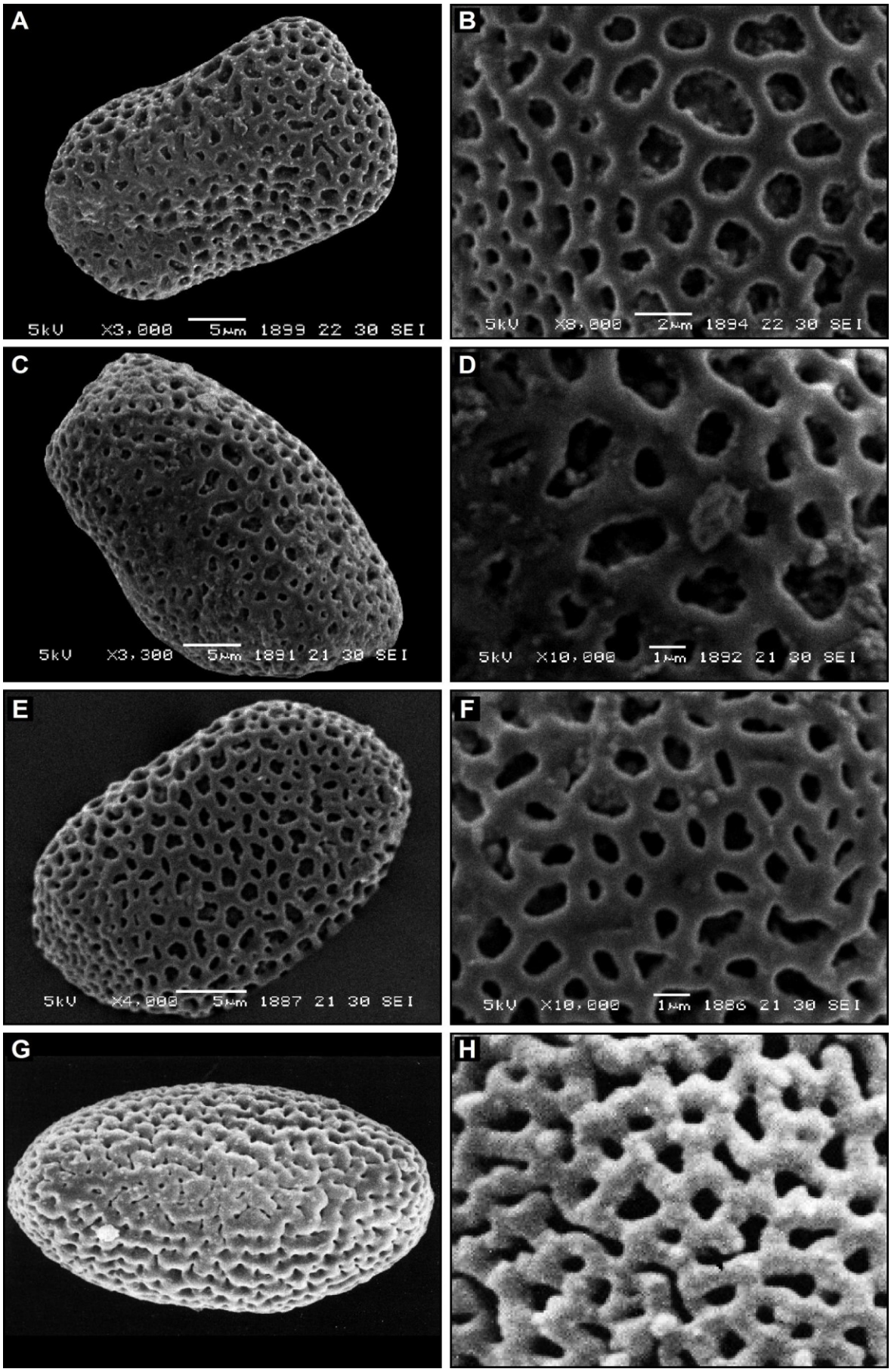

Fig. 2. Scanning Electron Micrographs (SEM): *Washingtonia filifera*: A, pollen, B, exine pattern. *Washingtonia robusta*: C, pollen; D, exine pattern. *Nannorrhops ritichiana*: E, pollen; F, exine pattern. *Sabal minor:* G, pollen; H, exine pattern (scale bar: D, H = 1 µm; B = 2 µm; A, C, E = 5 µm).

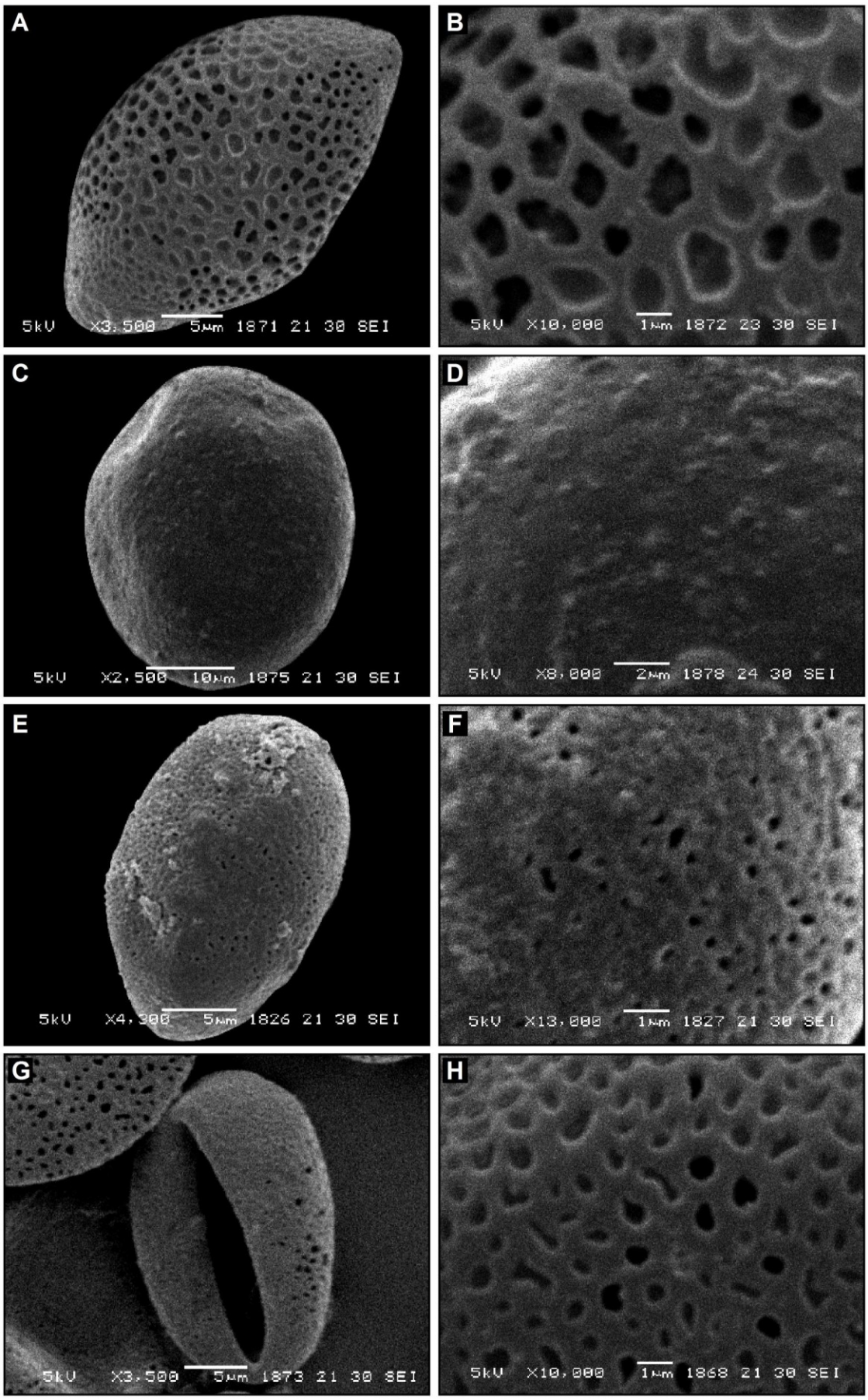

Fig. 3. Scanning Electron Micrographs (SEM): *Rhapis excelsa*: A, pollen; B, exine pattern. *Brahea brandegeei*: C, pollen; D, exine pattern. *Livistonia chinensis*: E, pollen; F, exine pattern. *Trachycarpus fortunei:* G, pollen; H, exine pattern (scale bar: B, F, H = 1µm; D, = 2µm; A, E, G = 5µm; C=10µm).

Table 3. General Pollen Characters of the subfamily Coryphoideae (excluding Phoeinaceae)

Name of taxa	Tribe	Length(µm)	Breadth(µm)	Colpus length (µm)	Aperture	Exine thickness (µm)	Tectum
Bismarkia nobilis	Borasseae	42.50 (45.95) 49.40 ± 0.769	26.00 (28.80) 31.60 ± 0.627	41.00(44.80)48.60 ± 0.798	monosulcate	1.0	rugulate or reticulate perforate
Borassus flabellifera	Borasseae	39.00(45.00) 51.00 ± 1.437	33.00 (36.50) 40.00 ± 0.755	37.50(43.65)49.80 ± 1.419	monosulcate	2.0-3.0	reticulate - verrucose
Hyphaene thebaica	Borasseae	34.00(36.00) 40.00 ± 0.672	31.00 (34.30) 38.00 ± 0.996	33.50 (36.25) 39.00 ± 0.566	monosulcate	2.0	reticulate - verrucose
Caryota urens	Caryoteae	13.50(20.35) 27.20 ± 1.772	16.20(25.40) 34.60 ± 2.361	13.00(19.70)26.40 ± 1.534	monosulcate	2 – 2.5	reticulate
Nannorrhops ritichiana	Corypheae	21.20(29.85)38.50 ± 1.899	28.30 (31.4) 34.50 ± 0.643	20.66(29.03) 37.40 ± 1.896	monosulcate	1.5	reticulate or foveolate - reticulate
Brahea brandegeei	Trachycarpeae	14.50 (16.55) 18.60 ± 0.502	20.00 (27.35) 34.20 ± 1.541	14.50 (16.25) 18.00 ± 0.430	monosulcate	1.0-1.5	perforate -reticulate
Livistonia chinensis	Trachycarpeae	15.10(19.70) 24.30 ± 1.052	23.68(28.94)34.20 ± 1.049	15.10(19.55)24.00 ± 0.944	monosulcate	1.0	perforate - reticulate
Pitrichardia beccariana	Trachycarpeae	19.50 (21.05) 22.60 ± 0.365	25.20 (30.45) 35.70 ± 1.068	19.00 (20.55) 22.10 ± 0.361	monosulcate	2.2	perorate- reticulate
Washingtonia filifera	Trachycarpeae	32.89(36.18) 39.47 ± 0.747	30.26(32.89)35.52 ± 0.569	32.60 (35.75) 38.90 ± 0.714	monosulcate	1.1	reticulate
Washingtonia robusta	Trachycarpeae	34.62(37.24) 39.86 ± 0.622	30.20(33.90)37.60 ± 0.741	33.25(36.32)39.40 ± 0.846	monosulcate	1.2	reticulate
Rhapis excelsa	Trachycarpeae	14.30 (16.65) 19.00 ± 1.35	21.80 (30.50) 38.30 ± 1.75	14.10 (15.80) 17.50 ± 1.22	monosulcate	1.9	reticulate
Rhapis multifida	Trachycarpeae	17.50 (19.95) 22.40 ± 0.762	27.20 (32.92) 38.65 ± 1.251	17.20 (19.50) 21.80 ± 0.499	monosulcate	2	reticulate
Trachycarpus fortunei	Trachycarpeae	14.80 (17.20)19.60 ± 0.560	19.40 (25.30) 31.00 ± 1.389	14.60 (17.10) 19.60 ± 0.523	monosulcate	1.8	coarsely reticulate
Sabal minor	Sabaleae	18.50 (19.95) 20.80 ± 0.269	27.60 (33.70) 40.00 ± 1.04	17.90(19.05) 20.20 ± 0.248	monosulcate	2.0	reticulate

Table 4. List of characters, scored for cluster analysis for the taxa of the subfamily Corphoideaeae in table 5

	Character description
1.	Length (µm)
2.	Breadth (µm)
3.	Exine thickness (µm)
4.	Aperture number
	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 (gemmae): Absent (0), Present (1)
	Exine pattern
16.	Reticulate: (including sub-reticulate): Absent (0), Present (1)
17.	Verrucose: Absent (0), Present (1)
18.	Perforate: Absent (0), Present (1)
19.	Punctate or foeveolate: Absent (0), Present (1)
20.	Rugulate: Absent (0), Present (1)
	Sexine pattern
21.	Muri simpli- baculate: Absent (0), Present (1)
22.	Muri simpli-duplibaculate: Absent (0), Present (1)
23.	Muri simpli-triplibaculate: Absent (0), Present (1)
24.	Muri simpli-multibaculate: Absent (0), Present (1)

Pollen description of the tribe Caryoteae

4. *Caryota* L.

Pollen elliptical or circular in polar view. Tectum finely clavate or less frequently spinose.

Caryota urens L. (Fig. 1A & B)

Pollen (13.50-) 20.35 (-27.20) µm in length and (16.20-) 25.40 (-34.60) µm in breadth, generally small, asymmetric. Aperture equal in length to the longer axis of pollen. Colpus (13.00-) 19.70 (26.40-) µm in length. Exine intectate, 2-2.5 µm thick. Tectum densely clavate-baculate, less frequently spinose or with protuberances; spines attached upper surface of foot layer.

Pollen description of the tribe Corypheae

5. *Nannorrhops* H. Wendl.

Pollen ellipsoidal or sub-circular in polar view, Tectum reticulate or foveolate-reticulate.

Nannorrhops ritichiana (Griff.) Aitc. (Fig. 2E & F)

Pollen (21.20-) 29.85 (-38.50) µm in length and (28.30-) 31.40 (- 34.50) µm in breadth, usually slightly asymmetric. Colpus (20.66-) 29.30 (-37.40) in length. Aperture margin psilate or scabrate. Exine tectate, 1.5 µm thick.Infratectum columellate. Muri simpli-dupli baculate.

Pollen description of the tribe Sabaleae

6. *Sabal* Adanson

Pollen elliptic in apertural view. Tectum finely reticulate.

Sabal minor (N.J. Jacq.) Persons (Fig. 2G & H)

Pollen (18.50-) 19.95 (-20.80) µm in length and (27.60-) 33.70 (-40.00) µm in breadth. asymmetric. Colpus (17.90-) 19.05 (-20.20) µm in length.Exine tectate, up to 2 µm thick. Infratectum columellate. Muri simple multibaculate.

Pollen description of the tribe Trachycarpeae

Pollen elliptic to subcircular in polar view. Size: Pollen (14.30-) 16.55 (-39.86) µm in length and (20.00-) 27.35 (-38.65) µm in breadth. Aperture moreor less equal in length to the longest axis, aperture membrane thin, smooth. Colpus (14.50-) 16.25 (-39.40) µm in length. Exine tectate, up to 1.5-2 µm thick. Infratectum collumellate.Tectum finely or densly reticulate, perforate to reticulate. Muri simple baculate to dupli or tripli to multibaculate.

The tribe comprises of 6 genera viz., *Brahea* Mart. ex Endl., *Livistona* R. Br., *Pritchardia* Labill., *Washingtonia* H. Wendl, *Rhapis* L. and *Trachycarpus* H. Wendl.

Key to the genera

1. + Pollen perforate ……...……...…….. **2**
- Pollen reticulate ……. …….. **4**

2. + Sexine simple. Pollen up to 18.60 µm in length. Minimum breadth is 20 µm..……...…..…. ***Brahea***
- Sexine tri-multibaculate. Pollen up to 24.30 µm in length. Minimum breadth is 36 µm ……...…… **3**

3. + Minimum pollen length is 15.10 µm. Exine 1.0 µm thick. Sexine tribaculate ……...…... ***Livistonia***
- Minimum pollen length is 19.50 µm. Exine more than 2 µm thick. Sexine multibaculate ...…...…….. ***Pritchardia***

4. + Pollen large, up to 40 µm in length ……..……..……..…….. ***Washingtonia***
- Pollen small, up to 22 µm in length ……...……...……..... **5**

5. + Breadth of pollen is more than 35 µm (i.e., 38.65 µm) ……...……...……. ***Rhapis***
- Breadth of pollen is less than 35 µm (i.e., 31.00 µm) ……..…… ***Trachycarpus***

Table 5. Data matrix of the taxa representing subfamily Coryphoideae scored for 24 characters present in the table 4.

Name of taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Bismarkia nobilis	40	28	1.0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0
Borassus flabellifer	40.8	36.5	2.5	1	1	0	0	1	0	0	0	1	1	0	1	1	1	0	0	0	1	0	0	0
Hyphaene thebaica	36	34.3	2.0	1	1	0	0	1	0	0	0	1	1	0	1	1	1	0	0	0	1	0	0	0
Caryota urens	20.3	25.4	2.0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0
Nannorrhops ritichiana	29.8	31.4	1.5	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0
Brahea brandegeei	16.6	27.3	1.2	1	1	0	0	1	0	0	0	1	1	0	0	1	0	1	0	0	1	0	0	0
Livistonia chinensis	19.7	28.9	1.9	1	1	0	0	1	0	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0
Pritchardia beccariana	21.0	30.4	2.2	1	1	0	0	1	0	0	0	1	1	0	0	1	0	1	0	0	0	1	0	0
Washingtonia filifera	36.2	32.9	1.1	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0
Washingtonia robusta	37.2	33.9	1.2	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0
Trachycarpus fortunei	17.2	25.31	1.8	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0
Rhapis excelsa	16.6	30.0	1.9	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0
Sabal minor	19.9	33.7	2.0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1

7. *Brahea* Mart. ex Endl.

Pollen elliptic in polar view. Tectum perforate.

Brahea brandegeei (Purpus) H.E. Moore (Fig. 3C & D)

Pollen (14.50-) 16.55 (-18.60) µm in length and (20.00-) 27.35 (-34.20) µm in breadth, asymmetric. Colpus (14.50-) 16.25 (-18.00) µm in length. Exine tectate, up to 2 µm thick. Muri simple multi-baculate.

8. *Livistonia* R. Br.

Pollen elliptic in apertural view. Tectum finely perforate to reticulate.

Livistonia chinensis (N. J. Jacquin) R. Br. ex Mart. (Fig. 3E & F)

Pollen (15.10-) 19.70 (-24.30) µm in length and (23.68-) 28.94 (-34.20) µm in breadth, asymmetric. Colpus (15.10-) 19.55 (-24.00) µm in length. Exine tectate, up to 2 µm thick. Muri simple multibaculate.

9. *Pritchradia* Labill.

Pollen elliptic in polar view. Tectum densely perforate to reticulate.

Pritchardia beccariana Rock (Fig. 1E & F)

Pollen (19.50-) 21.00 (-22.60) µm in length and (25.20-) 30.45 (-35.70) µm in breadth, asymmetrical. Colpus (19.00-) 20.55 (-22.10) µm in length. Exine tectate, up to 2.2 µm thick. Muri simple multibaculate.

10. *Washingtonia* H. Wendl.

Pollen elliptic in polar view. Tectum finely reticulate.

Washingtonia filifera (L. Linden) H. Wendl. (Fig. 2A & B)

Pollen (32.89-) 36.18 (-39.47) µm in length and (30.26-) 32.89 (-35.52) µm in breadth, Colpus (32.60-) 35.75 (-38.90) µm in length. Usually asymmetrical. Exine tectate, up to 1.1 µm thick. Muri simple multibaculate.

Washingtonia robusta H. Wendl. (Fig. 2C & D)

Pollen (34.62-) 37.24 (-39.86) µm in length and (30.20-) 33.90 (-37.60) µm in breadth, Colpus (33.25-) 36.32 (-39.40) µm in length. Exine tectate, up to 1.2 µm thick.

11. *Raphis* L.

Pollen elliptic in polar view. Tectum finely reticulate.

Rhapis excelsa (Thunb.) Henry ex Rehder (Fig. 3A & B)

Pollen (14.30-) 16.65 (-19.00) µm in length and (21.80-) 30.50 (-38.30) µm in breadth, asymmetric. Colpus (14.10-) 15.80 (-17.50) µm in length. Exine tectate, up to 2 µm thick. Muri simple multibaculate.

Rhapis multifida Burret

Pollen (-17.50) 19.95 (-22.40) µm in length and (27.20-) 32.92 (-38.65) µm in breadth, asymmetric. Colpus (17.20-) 19.50 (-21.80) µm in length. Exine tectate, up to 2 µm thick. Muri simple multibaculate.

12. *Trachycarpus* H. Wendl.

Pollen elliptic in polar view. Tectum finely reticulate.

Trachycarpus fortunei (Hook. f.) H. Wendl. (Fig. 3G & H)

Pollen (14.80-) 17.20 (-19.60) µm in length and (19.40-) 25.30 (-31.00) µm in breadth. Colpus (14.60-) 17.10 (-19.60) µm in length. Exine tectate, 1.8 µm thick. Muri simple multi-baculate.

Discussion

The pollen morphology of Coryphoideae is fairly uniform represented by monosulcate, perforate or finely reticulate pollen (excluding the Borossoid palm).The monosulcate aperture is considered to be a primitive character as pointed out by Wodehouse (1935), Kuprianova (1948), Thanikaimoni (1966) and traces its origin to the Palaeozoic Cordaitales. The present results are more or less in accordance with the earlier findings. For instance, Thanikaimoni (1970) described that all the coryphoid genera had similarity in their pollen morphological characters. Sowumni (1972) divided the tribe Borrisoideae into two groups on the basis of exine pattern whereas Coryphoideae and Phoenicoidae described as homogenous subfamilies. Similarly, Dransfield *et al.* (1990) classified the species of the coryphoid palms in the tribe Corypheae and Phoeniceae due to similarity in their pollen characters. Harley (1990) also mentioned that the monosulcate pollen type with perforate or reticulate tectum occurred throughout the entire family Palmae (including Coryphoideae). Thus, on the basis of the combinations of the following characters such as pollen size, exine thickness, exine pattern and the number of baculae, four pollen types are recognized in the subfamily Coryphoideae from the area under consideration. The pollen types are *Bismarkia*-type, *Borassus*-type, *Brahea*-type and *Sabal*- type. Similarly, the dendogram based on the 17 OTUs of pollen characters (subfamily Coryphoideae) also visibly indicates the existence of two major groups mainly on the basis of pollen size (Fig. 4).

Key to the pollen types

1. + Exine verrucose, up to 3µm thick, supra tectalgemmae present …….……..…….. ***Borassus*-type** (*Borassus flabellifer* & *Hyphene thebaica*)
   - Exine not as above, up to 2 µm thick, supra tectal gemmae absent ……..…….…… **2**
2. + Exine rugulate; up to 1 µm thick; pollen more than 45 µm in length ……..……..…….. ***Bismarkia*-type** (*Bismarkia nobilis*)
   - Exine not as above; pollen less than 40 µm in length ……..……..…….……..… **3**
3. + Exine mostly perforate ……..…….……. ***Brahea*–type** (*Brahea brandegeei*, *Livistonia chinensis* & *Pritchardia beccariana*)
   - Exine reticulate ……..……..…….……. ***Sabal*-type** (*Caryota urens, Nannorrhops ritichiana, Rhapis multifida, R. excelsa, Sabal minor, Trachycarpus fortunei, Washingtonia filifera* & *W. robusta*)

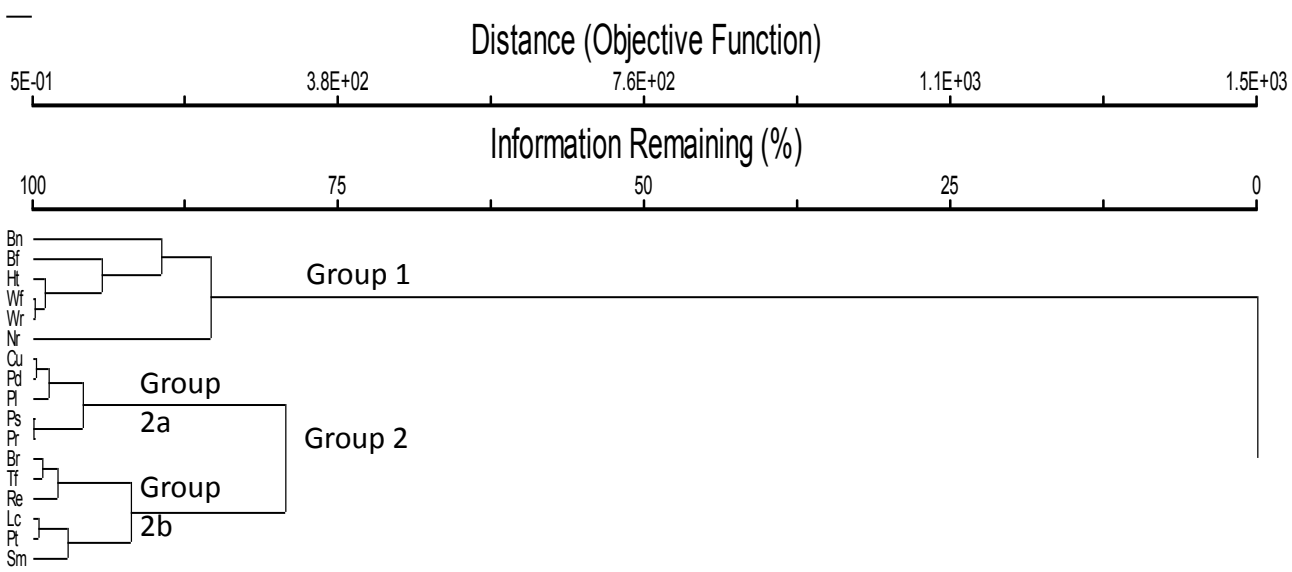

Fig. 4.Dendrogram obtained by Ward's cluster analysis, showing two groups of species belong to sub family Coryphoideae separated on the basis of different pollen characteristics.

Key to abbreviations: Bn = *Bismarkia nobilis,* Bf = *Borassus flabellifer,* Ht = *Hyphaene thebaica,* Cu = *Caryota urens,* Nr = *Nannorrhops ritichiana,* Bb = *Brahea brandegeei,* Lc = *Livistonia chinensis,* Pb = *Pritchardia beccariana,* Wf = *Washingtonia filiformis,* Wr = *Washingtonia robusta,* Tf = *Trachycarpus fortune,* Re = *Rhapis excels,* Pd = *Phoenix dactylifera,* Pl = *P. loureirii,* Ps = *P. sylvestris,* Pr = *P .robelli* and Sm = *Sabal minor.*

Considerable pollen variations have been found within the subfamily Coryphoideae with regard to exine pattern and pollen size. For instance, the tribe Borasseae is represented by two pollen types viz., *Borassus*-type and *Bismarkia*-type due to presence or absence of supra tectal gemmae as recorded in the literature also (Ferguson, 1986; Ferguson *et al*., 1987 and Ferguson & Harley, 1993). Furthermore, the Borassus-type is represented by 2 taxa representing different subtribes viz., Lataniinae (*Borassus flabellifer*) and Hyphaeninae (*Hyphene thebaica*). It is interesting to note that the pollen of *Hyphaene* are remarkably similar to *Borassus,* in shape, aperture, exine and particularly striking in the supratectal process (Fig. 1G-I), only a difference have been found in the quantitative characters i.e., size of pollen, columella and tectum (Table 3). The cluster analysis also shows the close association of both these genera (Fig. 4). Nevertheless, in terms of gross morphological characters both these taxa are not closely related as indicated by their inclusion in separate subtribes of the tribe Borraseae such as *Borassus* L. (Latannineae) and *Hyphaene* Gaertner (Hyphaeninae). The former genus has large and sessile female, symmetric fruits with apical stigmatic remains where as in the later genus the flower and fruits are different. Thus, the relationship between these genera clearly shows the lack of association between pollen morphology and gross morphology. However, remaining taxon of the borossoid palm (i.e., *Bismarkia nobilis*) falls in the Bismarkia-type, which occupy basal position in the dendogram and appeared to be partially linked with *Hyphaene* L. Palynologically, both genera shows visible difference mainly on the exine feature therefore fall into different pollen types (i.e., *Bismarkia*-type and *Borassus*-type). While, in terms of gross morphology both these genera (*Bismarkia* and *Hyphaene*) have small, globose, pedicellate female flowers and globule fruit with basal stigmatic remains (Dransfield & Uhl, 1998; Dransfield *et al*., 2008) thus belong to the same subtribe Hyphaenineae.

Furthermore, the Sabal-type is characterized by the presence of reticulate exine (Fig. 1A –B; Fig. 2A-H & Fig. 3A-B & G-H) and is represented by four tribes' viz., Caryotae, Corypheae, Sabaleae and Trachycarpae. The first three tribes are represented by a single species i.e., *Caryota urens*, *Nannorhops ritichiana* and *Sabal minor* respectively. All these taxa can be easily differentiated on the basis of pollen size and exine thickness (Table 3). Similarly, the dendogram also clearly indicates the position of the aforementioned taxa into three different groups due to variation in their pollen size such as *Nannorhops ritichiana* (Gp-1), *Caryota urens* (Gp 2a) and *Sabal minor* (Gp 2b) (see Fig. 4). The remaining tribe accommodates 5 taxa viz., *Rhapis multifida*, *R.excelsa*, *Trachycarpus fortunei*, *Washingtonia filifera* and *W. robusta.* These taxa are also differentiated on their pollen size. Cladistically, it can be proven that due to pollen size, the first three species show close linkage and occur in similar clade (Gp-2b) whereas the remaining taxa fall in the Gp-1 (Fig. 4). Besides, the rest of the species of the tribe Trachycarpeae viz., *Brahea brandegeei, Livistonia chinensis* and *Pritchardia beccariana* are included in the *Brahea*-type, due to presence of perforate exine pattern (Fig. 1C-D & Fig. 3C-F). The dendogram clearly indicates that these species appear in the same group i.e., Gp-2 (Fig. 4).

Hence, the foregoing discussion clearly indicates that monosulcate pollen are uniform throughout the subfamily Coryphoideae. Sowunmi (1972) and Dransfield *et al*. (1993) placed coryphoid palms in one group and described the homogenous nature of pollen except the tribe Borroseae. The present findings specify that the variations have been found in the pollen of the tribe Borassease as compared to the other tribes such as Caryotae, Corypheae, Trachycarpeae and Sabaleae. The present findings also in favour of the previous results and observe the almost same pollen morphological characters within the subfamily Coryphoideae. In some cases, pollen morphology shows a lack of correlation as in the tribe Borosseae (*Bismarkia* Hildebrandt. & H. Wendl.; *Borassus* L. and *Hyphaene* J. Gaertn.). The present findings are in accordance with some earlier reports such as Thanikaimoni (1970a); Sowunmi (1972); Ferguson & Harley (1993); Ferguson (1986); Ferguson *et al*. (1987) and Dransfield *et al*. (1990). Furthermore, on the basis of the combination of pollen characters, the studied taxa can be recognized easily at the generic level or higher level. However, at the specific level pollen data are not helpful because species of the representative genus have uniform pollen.

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