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POLLEN MORPHOLOGY OF DILLENIACEAE IN THAILAND

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Abstract

Pollen morphological studies on 18 species of Dilleniaceae from Thailand were conducted using dehydrated samples under a light microscope as well as scanning electron microscope. The pollen grains are monad, radially symmetrical, and isopolar. The pollen grains are small to medium in size with an equatorial diameter of 15.61-34.51 µm and polar diameter of 13.66-31.39 µm in the equatorial view. Most of the species of the genera Dillenia and Acrotrema are tricolpate, while *D. philippinensis* and *D. ovata* are tricolpate or occasionally tetracolpate, whereas the genus Tetracera is tricolporate. Variation is found in the shapes (oblate spheroidal, suboblate, subprolate, and prolate spheroidal), exine sculpturing (punctate, reticulate, regulate, and reticulate-verrucose), distance between the apices of two ectocolpi, colpus length, colpus width, porus length, porus width, and exine thickness. For numerical analysis, 13 qualitative characteristics and five quantitative characteristics were used for the principal component analysis and cluster analysis to determine the potential of the pollen morphological characteristics to be used to determine the species relationships. The results of the two numerical analysis methods showed that pollen morphological data could be used to classify the genera Acrotrema, Dillenia, and Tetracera into groups.

Key words: Dilleniaceae, Thailand, Numerical analysis, Pollen morphology.

Introduction

The Dilleniaceae is a family of angiosperms consisting of 10-14 genera and 500 species. It is distributed in the tropical and subtropical regions of the world (Horn, 2009). In Thailand, Dilleniaceae is represented by 3 genera and 17 species: 12 species of genus *Dillenia* L., four species of genus *Tetracera* L., and Acrotrema costatum (Hoogland, 1972; Na Songkhla & Chandraprasong, 2001). The intrafamilial relationships vary according to the data. Horn (2009) presented a new classification system based on molecular data. The Dilleniaceae were divided into four subfamilies: Delimoideae, Doliocarpoideae, Hibbertioideae, and Dellenioideae. While Hoogland (1952, 1953), Horn (2007), and Takhtajan (2009) divided the Dilleniaceae into two or four subfamilies using the characteristics of the stamen and wood anatomy. Hoogland (1952, 1953) and Takhtajan (2009) classified the Dilleniaceae into two subfamilies: Dellenioideae and Delimoideae. Horn (2007) divided the Dilleniaceae into four subfamilies: Delimoideae, Doliocarpoideae, Hibbertioideae, and Dellenioideae. According to the above data, the three genera of Dilleniaceae in Thailand are distributed among two subfamilies. The genera Dillenia and Acrotrema were placed in the subfamily Dellenioideae, while the genus Tetracera was placed in the subfamily Delimoideae. In several classification systems, the genera Dillenia, Tetracera, and Acrotrema were placed in the same group when using the characteristics of the anther dehiscence, superior ovary and sepal imbricate persistent (de Candolle, 1824; Bentham & Hooker, 1862; Hooker & Thomson, 1875; Gilg, 1895; Gilg & Werdermann, 1925; Hutchinson, 1964).

In the past, the study of plant taxonomy used the many morphological characteristics to identify, describe, and classify species of plants. At present, other knowledge is applied for the study of plant taxonomy, such as molecular biology, anatomy, chromosomes, embryology, and pollen morphology. Pollen morphology is used in plant taxonomy studies because the pollen grains have a variety of characteristics, i.e., size, shape, symmetry, aperture, exine structure, and exine sculpturing (Erdtman, 1952; Moore *et al*., 1991; Saensouk *et al*., 2015; Rashid *et al*., 2017). The pollen morphology of Dilleniaceae was briefly described at the species level by Erdtman (1952), Barth (1962), and Dickison (1967). They found that the shape of the pollen was oblate, spheroidal, or prolate. The aperture is triporate, tricolpate, tricolporate, and tetracolpate. The exine sculpturing is reticulate. Though the pollen morphology of Dilleniaceae has been published, but there is some incomplete data with no electronphotomicrographs. Dickison *et al*., (1982) completely described the pollen morphology of 38 species in the Dilleniaceae were light microscopy, scanning electron microscopy, and transition electron microscopy. The result showed that the pollen morphology of the 38 species of Dilleniaceae is as monads. Variations are found in the shape (spheroidal, suboblate, and subprolate) and aperture (tricolporate, tricolpate, and tetracolpate). The size of the pollen grains varies from 15-32 µm in the polar axis to 17-32 µm in the equatorial axis. The tectum is incomplete and punctate to reticulate. The palynological data of the family Dilleniaceae have never been described in detail for some of the characteristics, e.g., exine thickness, distance between apices of two ectocolpi, colpus length, colpus width, porus length, porus width,

and apocolpium index. Additionally, only a few of the species have been studied by researchers. Therefore, this study was designed to describe the pollen morphology of the Dilleniaceae from Thailand and to compare the pollen morphological data with the species relationship among the genera Acrotrema, Dillenia, and Tetracera.

Materials and Methods

Pollen morphology description: Pollen grains from 18 species of the Dilleniaceae were collected from the field in Thailand during 2013-2016 (Table 1). The pollen grains were examined using a light microscope (LM) and a scanning electron microscope (SEM). Voucher specimens were deposited in the herbarium at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand. Samples were prepared using a dehydrated alcohol series of 70%, 80%, 95%, and 100%. For LM analysis, samples were mixed with silicon oil (approximately 3-5 drops) in a vial, and then the samples were mounted on a glass slide and sealed with paraffin. The pollen grains were examined with an Axio LabA1 microscope with a digital photomicrograph system. The pollen types were measured to determine the polar axis (P), equatorial axis (E), size of aperture, and exine thickness using 30 pollen grains per sample for each specimen. For the SEM analysis, samples in absolute alcohol were dried on aluminium stubs with double-sided cellophane tape and coated with gold. The micrographs were taken using a SEM (JEOL: JSM 6460 LV) to determine the details of the exine sculpturing and aperture structure. The terminology classes follow Punt *et al*., (2007). Pollen shape classification follows Erdtman (1952) as based on the P/E ratio. Pollen size classes follow Erdtman (1952) as based on the length of the pollen grains longest axis.

Data analysis

The pollen morphology characters of each species were evaluated for morphometric analysis, comprising 13 quantitative and five qualitative characters (Table 2). The morphometric analysis was performed by factor analysis and cluster analysis. The analysis was conducted using Statistical Package for the Social Science (SPSS version 14 for Windows). Factor analysis was undertaken using the principal component analysis method (PCA). PCA was used to reduce the variables from a larger set into a smaller set. The result of the PCA is usually discussed in terms of the factor loading. The highest loadings are placed with the same factor and the PCA scatterplot can be developed from principal component I (factor I) and principal component II (factor II). Cluster analysis (CA) with the taxonomic distance was performed using the Euclidean distance and a phenogram was developed with the unweighted pair-group method using arithmetic average (UPGMA).

Table 1. **List of Dilleniaceae species in Thailand investigated in present study**.

Taxa	Location	Collection number	Remark
Acrotrema costatum Jack	Songkhla Province	S. Rakarcha 88	-
Dillenia aurea Sm.	Chaiyaphum Province	S. Rakarcha 60	-
D. excelsa (Jack) Martelli ex Gilg.	Trang Province	S. Rakarcha 83	-
D. grandifolia Wall. ex Hook.f. & Thomson	Trang Province	S. Rakarcha 90	-
D. hookeri Pierre	Ubon Ratchathani Province	S. Rakarcha 12	-
D. indica L.	Tak Province	S. Rakarcha 92	-
D. obovata (Blume) Hoogland	Trang Province	S. Rakarcha 89	-
D. ovata Wall. ex Hook.f. & Thomson	Yasothon Province	S. Rakarcha 11	-
D. parviflora Griff.	Chiang Rai Province	S. Rakarcha 71	-
D. pentagyna Roxd.	Chaiyaphum Province	S. Rakarcha 37	-
D. philippinensis Rolfe	Mahasarakham Province	S. Rakarcha 94	cultivated
D. reticulata King	Songkhla Province	S. Rakarcha 91	-
D. scabrella (D. Don) Roxb. ex Wall.	Loei Province	S. Rakarcha 69	-
D. suffruticosa (Griff.) Martelli	Bangkok Province	S. Rakarcha 26	-
Tetracera indica (Christm. & Panz.) Merr.	Phatthalung Province	S. Rakarcha 87	-
T. loureiri (Finet & Gagnep.) Pierre ex W. G. Craib	Ubon Ratchathani Province	S. Rakarcha 51	-
T. sarmentosa (L.) Vahl	Trang Province	S. Rakarcha 84	-
T. scandens (L.) Merr.	Songkhla Province	S. Rakarcha 85	-

Table 2. **Pollen morphological characters and character states used in numerical analysis of 18 species of Dilleniaceae in Thailand**.

No.	Characters	Character status
1	Polar axis (P)	Scale
2	Equatorial axis (E)	Scale
3	Polar axis/Equatorial axis ratio (P/E)	Scale
4	Exine thickness (Et)	Scale
5	Distance between apices of two ectocolpi (d)	Scale
6	Apocolpium index (d/D)	Scale
7	Colpus length (Clg)	Scale
8	Colpus width (Clt)	Scale
9	Colpus length/Colpus width (Clg/Clt)	Scale
10	Colpus length/Polar axis (Clg/P)	Scale
11	Porus lenght (Prg)	Scale
12	Porus width (Prt)	Scale
13	Porus lenght/Porus width (Prg/Prt)	Scale
14	Pollen shape (Ps)	Suboblate (0)
		Oblate spheroidal (1)
		Prolate-spheroidal (2)
		Subprolate (3)
15	Size (S)	Small (0)
		Medium (1)
16	Exine sculpturing types (Es)	Punctate (0)
		Reticulate (1)
		Regulate (2)
		Reticulate-verrucose (3)
17	Aperture type (A)	Tricolporate (0)
		Tricolpate (1)
		Tricolpate and tetracolpate (2)
18	P/E ratio	P/E ratio less than 1 (0)
		P/E ratio more than 1 (1)

Results

The pollen morphology of the family Dilleniaceae in Thailand was observed and analyzed under LM and SEM. The results are summarized in Tables 3 and 4. The general characteristic is that the pollan are monad, radially symmetrical, and isopolar. Detailed information of the taxa are described as follows:

Pollen size: The pollen grains of the studied species are small to medium in size (according to Erdtman (1952), who classified 10-25 µm as small pollen size and 25.1-50 µm as medium pollen size). Most of the studied species have small sized pollen grains (length of pollen less than 25 µm), except *D. aurea* and *D. philippinessis* that have medium sized pollen grains. The length of the polar axis (P) ranges from 13.66 µm in *D. reticulata* to 31.39 µm in

D. philippinensis. The length of the equatorial axis (E) ranges from 15.61 µm in D. parviflora to 34.51 µm in D. philippinensis.

Shape: The shape of the pollen in the equatorial view can be divided into four types based on the P/E ratio (according to Erdtman (1952)) as follows:

Type I: Comprises the species with the suboblate shape (100 x P/E ratio = 75.1-88), which includes *D. hookeri* (Fig. 1N), *D. indica* (Fig. 1Q), *D. reticulata* (Fig. 2Q), and *D. suffruticosa* (Fig. 3E).

Type II: Consists of the species with the oblate spheroidal shape (100 x P/E ratio = 88.1-100), which includes A. costatum (Fig. 1B), *D. aurea* (Fig. 1E), *D. excelsa* (Fig. 1H), *D. grandifolia* (Fig. 1K), *D. obovata* (Fig. 2B), D. ovata (Fig. 2E), *D. parviflora* (Fig. 2H), *D. pentagyna* (Fig. 2K), *D. philippinensis* (Fig. 2N), and *D. scabrella* (Fig. 3B).

Type III: Consists of the species with the prolate spheroidal shape (100 x P/E ratio = 100.1-114), which includes *T. loureiri* (Fig. 3K), *T. sarmentosa* (Fig. 3N), and *T. scandens* (Fig. 3Q).

Type IV: Comprises only *T. indica* with the subprolate shape (100 x P/E ratio = 114.1-133) (Fig. 3H).

Aperture: The results reveal that the pollen grains of the studied species are tricolpate, tetracolpate, and tricolporate. Most of the species assessed have tricolpate aperture, except *T. indica, T. loureiri, T. sarmentosa,* and *T. scandens* that have a tricolporate aperture. The porus shape of the tricolporate aperture is ovoid. The porus length varies from 2.41 µm (*T. sarmentosa*) to 2.79 µm (*T. loureirii*), and the porus width varies from 1.45 µm (*T. scandens*) to 1.67 µm (*T. sarmentosa*). Variations in the aperture are found in *D. ovata* (Fig. 2D) and *D. philippinensis* (Fig. 2M) that have tricolpate and occasionally tetracolpate apertures. The colpus membranes of all the samples are covered with large and irregular flecks. The length of the aperture varies from 4.60 µm in A. costatum to 13.73 µm in *T. indica*, and the width of the aperture varies from 1.72 µm in T. indica to 3.26 µm in *D. philippinensis*. The distance between the apices of the two ectocolpi varies from a minimum of 4.52 µm in *T. sarmentosa* to a maximum of 15.81 µm in *A. costatum*.

Table 3. **Pollen morphological characteristic of Dilleniaceae in Thailand**.

Species	Shape	Size	Aperture	Exine sculpturing
Acrotrema costatum	Oblate spheroidal	Small	Tricolpate	Punctate
Dillenia aurea	Oblate spheroidal	Medium	Tricolpate	Reticulate
D. excelsa	Oblate spheroidal	Small	Tricolpate	Reticulate
D. grandifolia	Oblate spheroidal	Small	Tricolpate	Reticulate
D. hookeri	Suboblate	Small	Tricolpate	Reticulate
D. indica	Suboblate	Small	Tricolpate	Punctate
D. obovata	Oblate spheroidal	Small	Tricolpate	Regulate
D. ovata	Oblate spheroidal	Small	Tricolpate, Tetracolpate	Reticulate
D. parviflora	Oblate spheroidal	Small	Tricolpate	Punctate
D. pentagyna	Oblate spheroidal	Small	Tricolpate	Reticulate-verrucose
D. philippinensis	Oblate spheroidal	Medium	Tricolpate,Tetracolpate	Punctate
D. reticulata	Suboblate	Small	Tricolpate	Reticulate
D. scabrella	Oblate spheroidal	Small	Tricolpate	Reticulate-verrucose
D. suffruticosa	Suboblate	Small	Tricolpate	Punctate
Tetracera indica	Subprolate	Small	Tricolporate	Punctate
T. loureiri	Prolate-spheroidal	Small	Tricolporate	Punctate
T. sarmentosa	Prolate-spheroidal	Small	Tricolporate	Punctate
T. scandens	Prolate-spheroidal	Small	Tricolporate	Punctate

Table 4. Pollen morphometric characteristics and measurements of Dilleniaceae in Thailand: numbers refer to minimum-maximum (mean ± standard deviation), polar axis (P), equatorial axis (E), polar axis/equatorial axis (P/E) ratio, exine thickness (Et), distance between apices of two ectocolpi (d), apocolpium index (d/D), colpus length (Clg), colpus width (Clt), colpus length/colpus width (Clg/Clt), colpus length/polar axis (Cl/P), porus length (Prg), porus width (Prt), porus length/porus width (Prg/Prt), and characteristic not found (-).

Species	P (µm) (Mean ± S.D.)	E=D (µm) (Mean ± S.D.)	P/E ratio	Et (µm) (Mean ± S.D.)	d	d/D	Clg (µm) (Mean ± S.D.)	Clt (µm) (Mean ± S.D.)	Clg/Clt	Clg/P	Prg (µm) (Mean ± S.D.)	Prt (µm) (Mean ± S.D.)	Prg/Prt
Acrotrema costatum	18.21-24.34 (20.77±1.63)	20.65-26.61 (23.28±1.26)	0.892	0.72-1.39 (1.01±0.19)	12.42-17.27 (15.81±0.99)	0.68	3.60-5.60 (4.60±0.65)	1.88-3.67 (2.68±0.67)	1.72	0.22	-	-	-
Dillenia aurea	19.84-26.68 (22.85±1.72)	23.18-28.98 (25.93±1.63)	0.881	0.80-1.87 (1.40±0.31)	9.47-14.69 (11.75±1.64)	0.45	7.00-11.16 (9.14±1.65)	2.33-3.75 (3.04-0.46)	3.01	0.40	-	-	-
D. excelsa	14.93-19.54 (16.78±1.06)	17.19-20.82 (18.79±1.04)	0.893	0.68-1.24 (0.91±0.13)	7.10-12.01 (9.67±1.76)	0.51	5.94-11.30 (8.37±1.54)	1.31-3.21 (1.98±0.54)	4.23	0.49	-	-	-
D. grandifolia	14.16-19.49 (15.98±1.03)	14.92-21.24 (17.45±1.43)	0.916	0.70-1.28 (0.99±0.13)	9.02-10.92 (10.13±0.57)	0.58	3.94-7.12 (5.81±1.29)	1.78-2.37 (2.02±0.21)	2.87	0.36	-	-	-
D. hookeri	15.18-20.38 (17.30±1.43)	18.49-24.79 (20.86±1.41)	0.829	0.69-1.39 (0.98±0.17)	7.50-16.02 (11.12±2.68)	0.53	10.26-12.35 (11.49±0.78)	2.50-3.33 (2.84±0.29)	4.05	0.66	-	-	-
D. indica	16.84-20.21 (18.21±0.73)	19.59-23.74 (21.26±0.94)	0.857	0.69-1.39 (0.98±0.18)	10.19-13.8 (11.79±0.92)	0.55	5.79-8.26 (6.95±0.82)	2.50-3.97 (2.89±0.46)	2.40	0.38	-	-	-
D. obovata	17.62-22.16 (19.38±1.08)	18.83-24.66 (21.05±1.35)	0.921	0.66-1.33 (1.01±0.18)	9.21-14.10 (11.79±1.37)	0.56	7.68-12.56 (9.79±1.65)	1.46-2.08 (1.79±0.20)	5.46	0.51	-	-	-
D. ovata	18.08-21.43 (19.26±0.83)	19.24-21.95 (20.43±0.74)	0.943	0.66-1.17 (0.85±0.12)	8.33-13.12 (9.50±1.22)	0.47	8.02-10.66 (9.40±0.96)	1.18-2.67 (2.21±0.46)	4.25	0.49	-	-	-
D. parviflora	13.01-15.89 (14.48±0.83)	14.59-17.64 (15.61±0.72)	0.928	0.74-1.66 (1.17±0.24)	6.20-12.45 (8.21±2.01)	0.53	7.18-11.00 (9.35±1.23)	1.65-2.10 (1.92±0.13)	4.87	0.65	-	-	-
D. pentagyna	13.88-23.65 (17.63±2.24)	15.29-23.77 (18.00±1.92)	0.979	0.68-1.46 (1.04±0.23)	5.26-12.24 (9.53±2.03)	0.53	8.20-14.21 (10.01±2.21)	1.74-2.75 (2.17±0.33)	4.61	0.57	-	-	-
D. philippinensis	29.54-33.50 (31.39±1.22)	31.64-36.74 (34.51±1.45)	0.909	0.94-1.64 (1.26±0.20)	12.61-15.21 (13.61±1.05)	0.39	7.75-12.87 (10.27±1.92)	2.84-4.24 (3.26±0.47)	3.15	0.32	-	-	-
D. reticulata	12.57-15.25 (13.66±0.67)	13.99-17.38 (15.64±0.86)	0.873	0.70-1.36 (0.95±0.19)	6.84-10.80 (9.31±1.01)	0.59	3.36-6.25 (5.31±0.84)	1.55-2.01 (1.82±0.16)	2.92	0.39	-	-	-
D. scabrella	17.51-21.42 (18.87±0.96)	18.14-22.02 (20.05±0.98)	0.941	1.04-2.32 (1.58±0.25)	6.93-8.68 (7.64±0.65)	0.38	6.07-9.47 (7.87±1.26)	1.56-2.23 (1.82±0.24)	4.32	0.41	-	-	-
D. suffruticosa	17.60-20.79 (19.19±0.72)	20.04-25.01 (23.36±0.99)	0.821	0.66-1.53 (1.00±0.18)	8.33-12.17 (10.65±1.57)	0.46	8.98-11.48 (10.56±0.75)	1.65-3.67 (2.39±0.67)	4.42	0.55	-	-	-
Tetracera indica	16.96-20.90 (19.17±0.80)	11.86-17.96 (16.06±1.43)	1.194	0.51-1.29 (0.91±0.17)	3.33-5.52 (4.58±0.65)	0.23	12.03-15.29 (13.73±1.24)	1.33-1.98 (1.72±0.19)	7.98	0.71	2.25-2.89 (2.55±0.31)	1.32-1.87 (1.60±0.19)	1.59
T. loureiri	16.42-19.42 (18.09±0.57)	15.08-17.85 (16.79±0.58)	1.077	0.74-1.23 (1.03±0.11)	4.47-6.40 (5.18±0.50)	0.29	10.50-14.07 (12.53±1.20)	1.45-1.95 (1.74±0.16)	7.20	0.74	2.55-2.95 (2.79±0.16)	1.45-1.78 (1.57±0.13)	1.78
T. sarmentosa	15.11-17.66 (16.41±0.52)	14.53-17.34 (15.89±0.53)	1.033	0.49-1.41 (0.92±0.17)	3.29-5.26 (4.52±0.52)	0.28	11.27-15.13 (13.25±1.20)	1.45-2.20 (1.74±0.26)	7.61	0.81	1.85-2.87 (2.41±0.40)	1.45-1.85 (1.67±0.14)	1.44
T. scandens	14.68-19.28 (16.97±0.98)	13.77-18.18 (16.04±0.83)	1.058	0.71-1.33 (0.94±0.71)	4.11-6.23 (4.97±0.69)	0.30	10.77-13.74 (12.30±0.91)	1.65-2.44 (1.98±0.33)	6.21	0.72	1.95-2.78 (2.54±0.34)	1.45-2.20 (1.45±2.20)	1.75

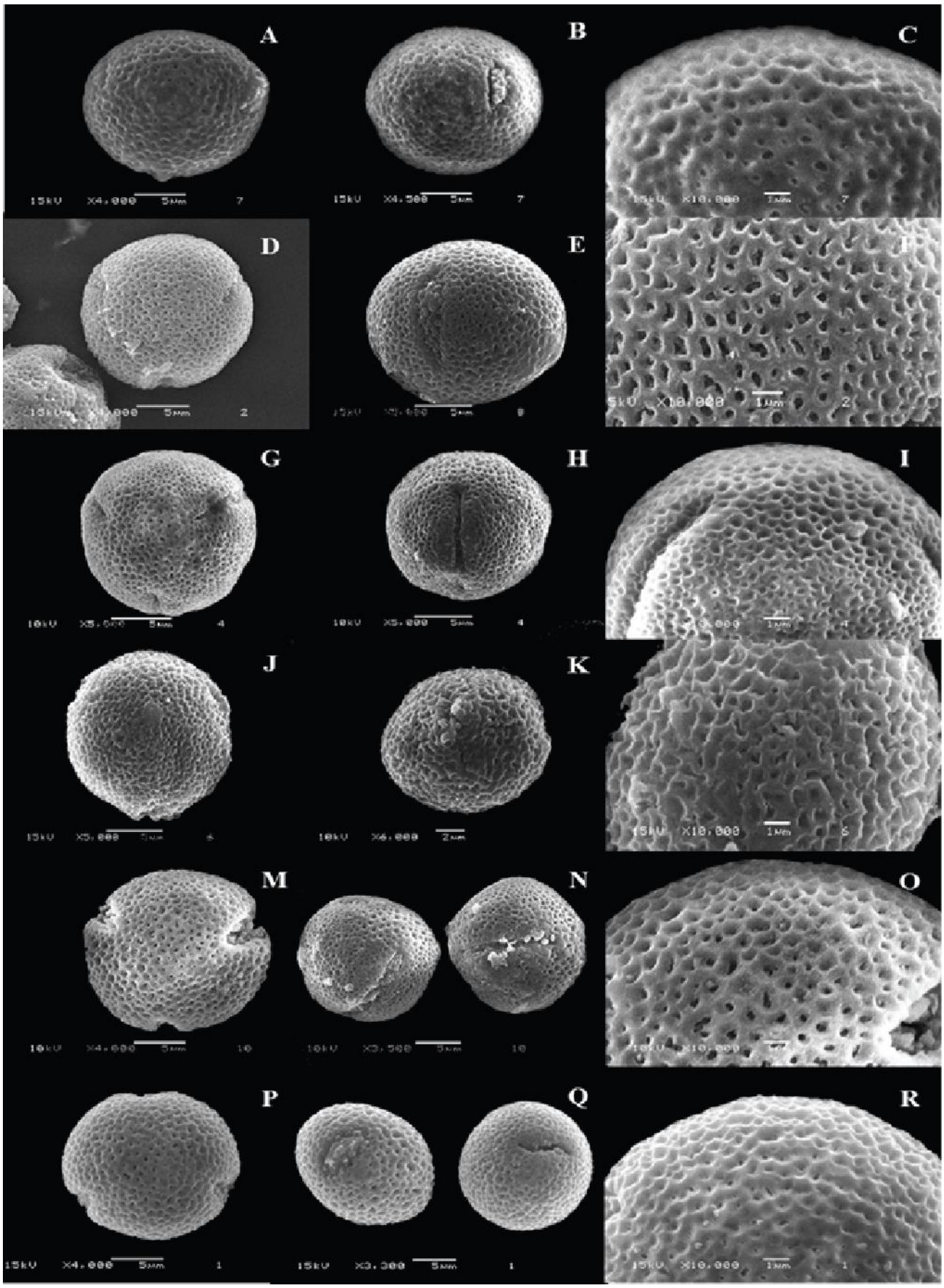

Fig. 1. SEM micrographs of pollen grains of Dilleniaceae: *A. costatum* A-C, *D. aurea* D-F, *D. excelsa* G-I, *D. grandifolia* J-L, *D. hookeri* M-O, and *D. indica* P-R; polar view A, D, G, J, M, and P; equatorial view B, E, H, K, N, and Q; and exine sculpturing C, F, I, L, O, and R.

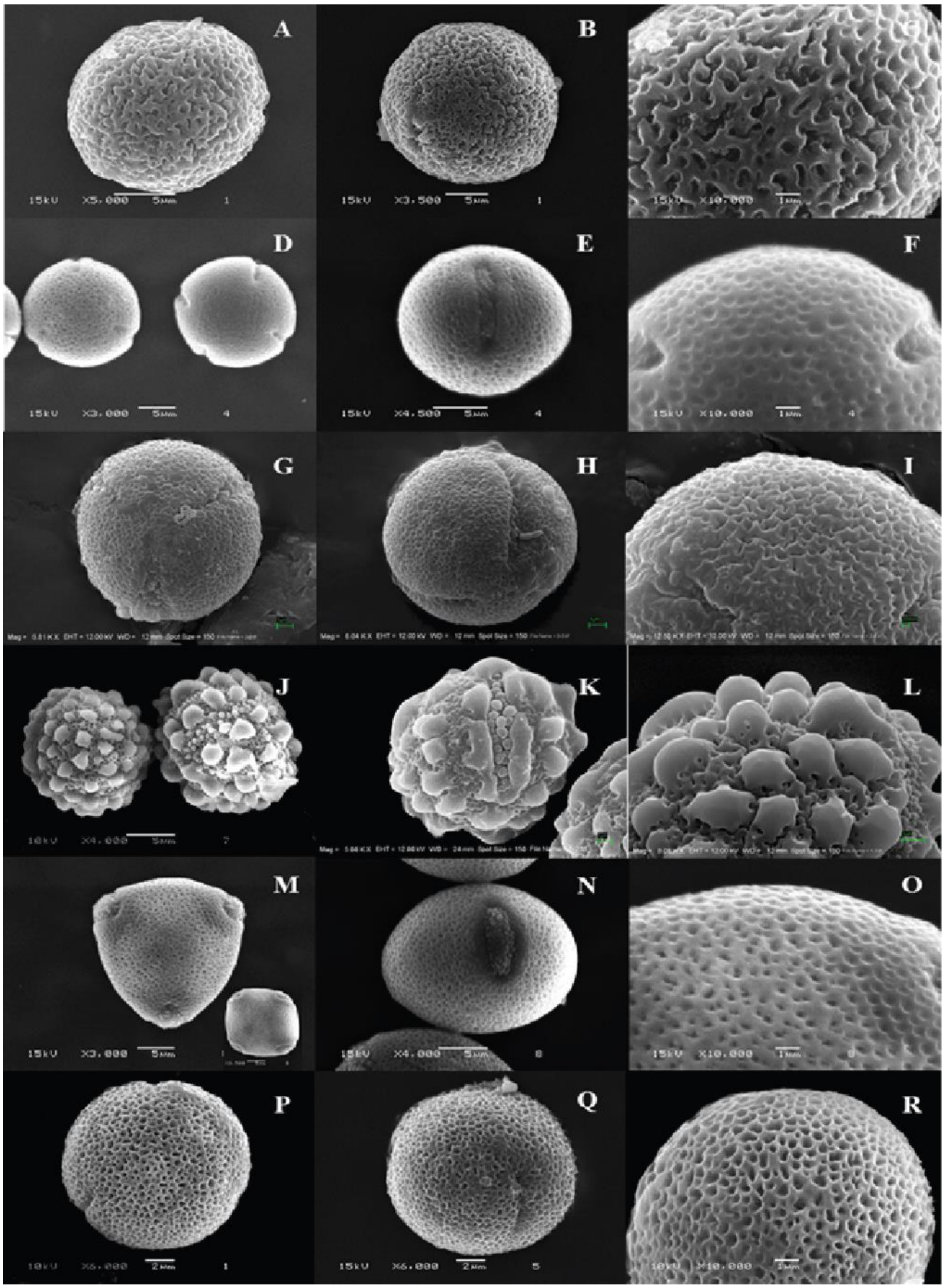

Fig. 2. SEM micrographs of pollen grains of Dilleniaceae: *D. obovata* A-C, *D. ovata* D-F, *D. paviflora* G-I, *D. pentagyna* J-L, *D. philippinensis* M-O, and *D. reticulata* P-R; polar view A, D, G, J, M, and P; equatorial view B, E, H, K, N, and Q; and exine sculpturing C, F, I, L, O, and R.

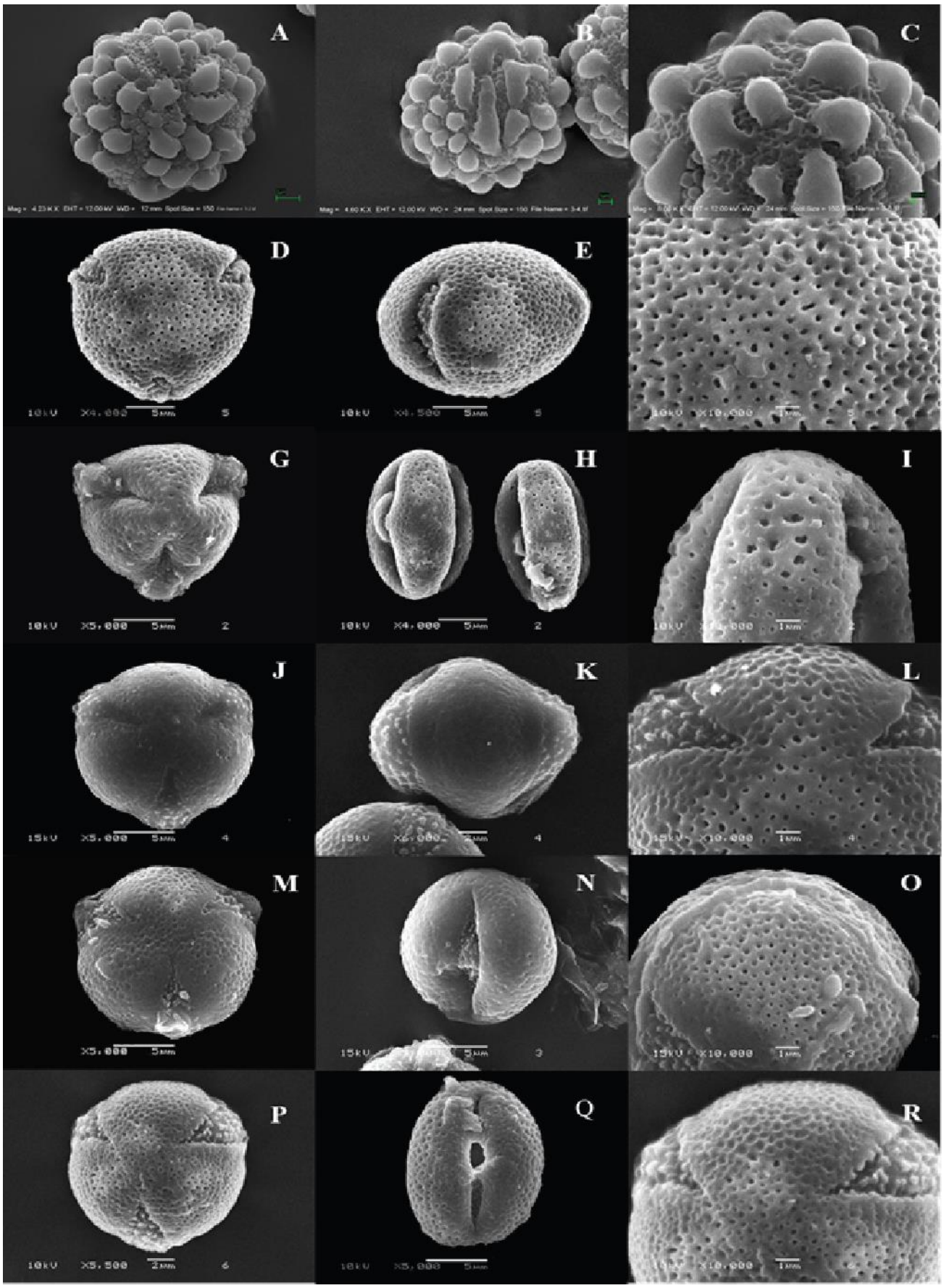

Fig. 3. SEM micrographs of pollen grains of Dilleniaceae: *D. scabrella* A-C, *D. suffruticosa* D-F, *T. indica* G-I, *T. loureiri* J-L, *T. sarmentosa* M-O, and *T. scandens* P-R; polar view A, D, G, J, M, and P; equatorial view B, E, H, K, N, and Q; and exine sculpturing C, F, I, L, O, and R.

Exine sculpturing: The exine thickness of all the samples varies from a minimum of 0.85 µm in *D. ovata* to a maximum of 1.58 µm in *D. scabrella*. The exine sculpturing can be divided into four types based on the tectal perforation as follows:

Type I: Comprises the species with the punctate perforation type, in which the tectal perforations have a length or diameter of less than 1 µm. This type is observed in *A. costatum* (Fig. 1C), *D. indica* (Fig. 1R), *D. parviflora* (Fig. 2I), *D. philippinensis* (Fig. 2O), *D. suffruticosa* (Fig. 3F), *T. indica* (Fig. 3I), *T. loureiri* (Fig. 3L), *T. sarmentosa* (Fig. 3O), and *T. scandens* (Fig. 3R).

Type II: Consists of the species with the reticulate perforation type, in which the tectal perforations or lumina are wider than 1 µm. The network-like pattern of exine sculpturing consists of *D. aurea* (Fig. 1F), *D. excelsa* (Fig. 1I), *D. grandiolia* (Fig. 1L), *D. hookeri* (Fig. 1O), *D. ovata* (Fig. 2F), and *D. reticulata* (Fig. 2R).

Type III: Consists of the species with the rugulate perforation type, in which sexine elements are elongated more than 1 µm. This type is recorded only in *D. obovata* (Fig. 2C).

Type IV: Exine have the reticulate perforation type and its sexine is wart-like, more than 1 µm, and the base is wider than the height. It is called the reticulate-verrucose perforation type, which is found in *D. pentagyna* (Fig. 2L) and *D. scabrella* (Fig. 3C).

Principal component analysis (**PCA**): The results of the factor loading scores and eigen analysis based on 18 characters related to the 18 species are presented in Tables 5 and 6. Three components explain 87.39% of the total variance in the pollen morphological character. The first principle component accounts for 60.51% of the total variance and is dominated by the apocolpium index (d/D), porus width (Prt), aperture type (A), polar axis/equatorial axis (P/E) ratio, porus length (Prg), porus length/porus width (Prg/Prt), colpus length/colpus width (Clg/Clt), polar axis/equatorial axis (P/E), pollen shape (Ps), colpus length (Clg), distance between apices of two ectocolpi (d), and colpus length/polar axis (Clg/P). The second principle component accounts for 18.75% of the total variance and is mainly associated with the polar axis (P), equatorial axis (E), size (S), and colpus width (Clt). The third principle component describes 8.13% of the total variance and is influenced by the exine sculpturing types (Es) and exine thickness (Et).

The PCA scatter plot is created by factor score 1 (principle component 1) and factor score 2 (principle component 2). Thai Dilleniaceae species are placed into three groups as shown in Figure 4. *D. philippinensis* is grouped in the first group. The second group comprises 13 species: *A. costatum, D. aurea, D. excelsa, D. grandifolia, D. hookeri, D. indica, D. obovata, D. ovata, D. parviflora, D. pentagyna, D. reticulata, D. scabrella,* and *D. suffruticosa*. The third group consists of the species of the genus Tetracera: *T. indica, T. loureiri, T. sarmentosa,* and *T. scandens*.

Table 5. **Factor loadings explaining relationships of variables and showing most intrinsic characters for separation of studied Dilleniaceae species**.

Variable	Factor loadings		
	Principal components		
	1	2	3
d/D	-0.94	-0.15	
Prt	0.93	-0.16	0.26
A	0.93	-0.16	0.27
P/E retio	0.93	-0.16	0.27
Prg	0.93	-0.16	0.26
Prg/Prt	0.93	-0.16	0.27
Clg/Clt	0.90	-0.29	
P/E	0.90	-0.18	
Ps	0.88		
Clg	0.83		
d	-0.83	0.45	0.10
Clg/P	0.75	-0.39	0.14
P		0.95	
E	-0.31	0.92	
S		0.91	-0.15
Clt	-0.44	0.78	0.24
Es	-0.29	-0.24	-0.83
Et		0.49	-0.72

Key to abbreviations: apocolpium index (d/D), porus width (Prt), aperture type (A), polar axis/equatorial axis (P/E) ratio, porus length (Prg), porus length/porus width (Prg/Prt), colpus length/colpus width (Clg/Clt), pollen shape (Ps), colpus length (Clg), distance between apices of two ectocolpi (d), colpus length/polar axis (Clg/P), polar axis (P), equatorial axis (E), size (S), colpus width (Clt), exine sculpturing types (Es), and exine thickness (Et).

Table 6. Eigen analysis of correlation matrix.

Component	Eigenvalues		
	Total	**% of Variance**	**Cumulative %**
1	10.89	60.51	60.51
2	3.38	18.75	79.26
3	1.46	8.13	87.39

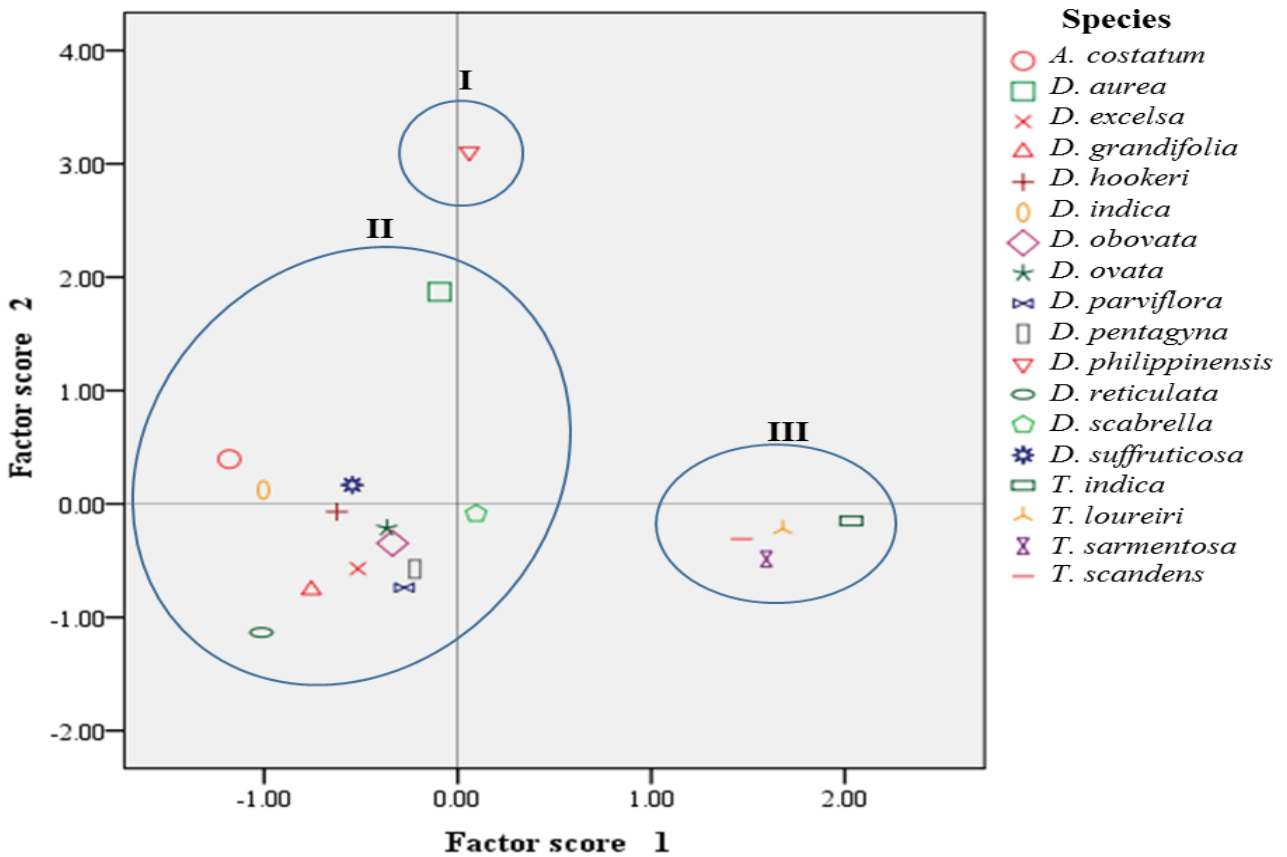

Fig. 4. PCA scatterplot of Dilleniaceae species in Thailand based on pollen morphological characters.

Cluster analysis: The UPGMA dendrogram of the cluster analysis is shown in Figure 5. The dendrogram grouped 18 species into three major clusters based on the pollen morphological characters. The first cluster consisted of *D. philippinensis*. The second cluster consisted of *A. costatum*, *D. aurea*, *D. excelsa*, *D. grandifolia*, *D. hookeri*, *D. indica*, *D. obovata*, *D. ovata*, *D. parviflora*, *D. pentagyna*, *D. reticulata*, *D. scabrella*, and *D. suffruticosa*. The third cluster included four species of the genus Tetracera: *T. indica*, *T. loureiri*, *T. sarmentosa*, and *T. scandens*.

Discussion

These results revealed the pollen morphological characteristics within the family Dilleniaceae in Thailand including details of the size, shape, aperture, and exine wall. For pollen size, small sizes are dominant in 16 species of Thai Dilleniaceae species, whereas the medium size is recorded in *D. aurea* and *D. philippinensis*. These pollen morphological data are in agreement with previous studies on some species by Barth (1962), Erdtman (1952), Dickison (1967), and Dickison et al. (1982). The pollen size can be used to separate some species, especially *D. philippinensis* for the largest (polar axis ranged 29.54-33.50 µm) and *D. parviflora* for the smallest pollen size (polar axis ranged 13.01-15.89 µm).

In our study, the pollen grains vary in shape from oblate spheroidal, suboblate, subprolate, to prolate spheroidal. The findings are consistent with some species from the studies of Dickison (1967) and Dickison *et al.* (1982), in which they reported that the pollen shapes of the genera Acrotrema, Dillenia, and Tetracera varied, as follows: the genus Acrotrema are oblate spheroidal; oblate, oblate spheroidal, or spheroidal were recorded for the genus Dillenia; and spheroidal, prolate spheroidal, or prolate were recorded in the genus Tetracera. Barth (1962) pointed out that the pollen shape of the genus Tetracera was subprolate. Erdtman (1952) reported the pollen shapes of *D. ovata* and *T. scandens* as being oblate spheroidal and prolate spheroidal, respectively, and these data were consistent with the present study. In addition,

the present study reports an additional pollen shape of suboblate as found in several species of the genus Dillenia.

Our results show that the aperture of the pollen grain can be used for grouping the Dilleniaceae in Thailand into two groups viz., the compound aperture (tricolporate) including genus Tetracera and tricolpate with elongated apertures representing *A. costatum* and the genus Dillenia. Our results and those of Dickison *et al*., (1982) confirm that tricolporate pollen represents the basic condition of Tetracera, as well as Curatella, Davilla, Doliocarpus, and Pinzona, while tricolpate pollen are the basic condition of Acrotrema and Dillenia. Dickison (1967) reported very short length in the colpus of *D. indica* and *D. philippinensis* which were classified as porate. The present study and a previous study by Dickison *et al*., (1982) confirm that the apertures of *D. indica* and *D. philippinensis* are colpate based on SEM photographs. Previously, Dickison (1967) first observed that in some species of the genus Dillenia the aperture is tricolplate while occasionally tetracolpate within single species. Our study confirms that the tetracolpate form can be found occasionally with the tricolpate form in some species of the genus Dillenia.

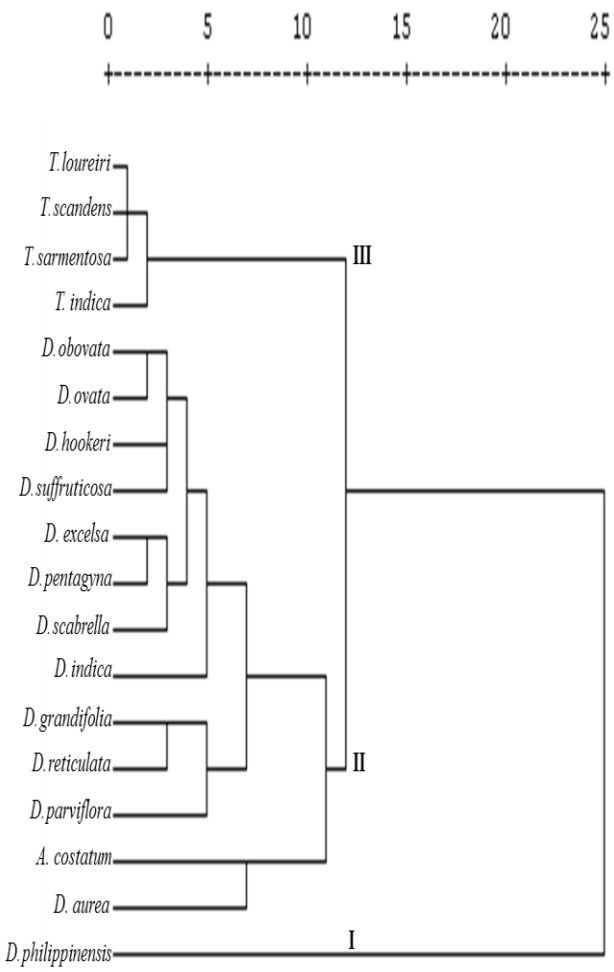

Fig. 5. UPGMA clustering of 18 OTUs based on Euclidean distance matrix calculated between means of eight quantitative and five qualitative pollen morphological characters of Dilleniaceae in Thailand.

The present observation of the exine sculpturing classified it as punctate, reticulate, regulate, and reticulate-verrucose. Dickison *et al*., (1982) pointed out that in some species of the genus Dillenia, such as *D. pentagyna*, the tactum consisted of granules on the surface of the exine and exine sculpturing, which was classified as punctate-striate. In the present study, the exine sculpturing of *D. pentagyna* is classified as reticulate-verrucose as the outer wall of the lumina is wider than 1 µm and combined with a wart-like surface. In addition, those characteristics are found in *D. scabella*. Punt *et al*., (2007) point out that the differentiation of the striate and verrucose by striate is the sculpturing elements that are parallel and separated by grooves, while, for verrucose it is sexine elements that contain wart-like structures, more than 1 µm, and where the base is wider than the height. The result from the present study and Dickison *et al*., (1982) differs from Barth (1962), Erdtman (1952), and Dickison (1967) reported that in all cases the genus Tetracera had exine sculpturing that is reticulate, but the present study and the study of Dickison *et al*., (1982) from SEM observations point out that the exine sculpturing of the genus Tetracera is punctate. Exine sculpturing cannot be utilized in classifying the Dilleniaceae into tribes or subfamilies because of an overlap between genera.

The results of the PCA scatter plot and UPGMA clustering based on the pollen morphological characteristics are similar. The PCA scatter plot is classified into three groups, which are all the same as in the UPGMA dendrogram. The species of the genus Tetracera in Thailand are placed in group three, which is separated from the other groups. The prominent pollen morphological characters of the genus Tetracera are tricolporate, d/D ratio from 0.2-0.3, P/E more than 1, Clg/Clt more than 6, Clg/P more than 0.7, subprolate, and prolate-spheroidal in shape. The remaining group consisted of the genera Dillenia and Acrotrema, in which all groups had tricolpate aperture, d/D ratio more than 0.3, P/E less than 1, Clg/Clt less than 6, Clg/P less than 0.7, oblate spheroidal, and suboblate in shape. *D. philippinensis* is separated from the other groups due to its polar axis, equatorial axis, and colpus width being larger than the other species. The present observations show that pollen morphological characteristics, such as shape, aperture, d/D ratio, P/E ratio, Clg/Clt ratio, and Clg/P ratio, support a relationship between the genera Acrotrema, Dillenia, and Tetracera.The evidence from pollen morphology supports a close link between Acrotrema and Dillenia, while Tetracera is placed elsewhere. The relationship of Acrotrema, Dillenia, and Tetracera is supported by the studies of Hoogland (1952, 1953), Takhtajan (2009), and Horn (2007), who all placed Dillenia and Acrotrema in the same group based on the morphological characteristic and wood anatomy as follows: anthers linear or oblong, loculi very rarely slightly divergent, and lateral pitting scalariform-transitional; while, the genus Tetracera was classified into the other group based on the morphological characteristic and anatomy as follows: anthers short,

loculi divergent, separated at the base by the thickened connective, and lateral pitting opposite-transitional. While Horn (2009) arranged the genera Dillenia and Acrotrema in the same group and classified the genus Tetracera in another group based on molecular data.

In the present work, the result shows additional characteristics for the first time for the Dilleniaceae such as exine thickness, colpus length, colpus width, ratio between colpus length and polar axis, ratio between colpus length and colpus width, porus length, porus width, ratio between porus length and porus width, distance between apices of two ectocolpi, and apocolpium index. The above characteristics of the pollen are very important for grouping the Dilleniaceae in Thailand, such as the apocolpium index of the genus Tetracera ranged from 0.20-0.30, while for the genera Acrotrema and Dillenia it ranged from 0.40-0.70.

Conclusion

The present observation provides a definite clue to the relationships of the Acrotrema, Dillenia, and Tetracera. We suggest that detailed analysis of the pollen morphology is extremely important for understanding the taxa. The pollen morphology data cannot be used for species identification due to the variation within and overlap between species. The elongated aperture covered with large irregular flecks and tri-aperture represents the basic characteristic in the Dilleniaceae. Moreover, using the pollen morphological characteristic in combination with other characters, such as anatomy and morphology, may help in the identification of species and problematic taxa.

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