

POLLEN FLORA OF PAKISTAN – LXVII: ACANTHACEAE

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Abstract

Pollen morphology of 30 species of the family Acanthaceae belonging to 11 genera has been investigated using light and scanning electron microscope. Acanthaceae is a eurypalynous family. Pollen are usually radially symmetrical, isopolar, sub-prolate to prolate rarely prolate-spheroidal or sub-oblate to oblate-spheroidal, generally tricolporate or heterocolporate rarely colpate or porate. Exine ornamentation varies from medium to coarse reticulate, or often lopho-reticulate with luminae perforated to baculate or scabrate. On the basis of apertural type, exine ornamentation and colpal membrane eight distinct pollen types have been recognized viz., Pollen type-I: *Barleria cristata*-type, Pollen type-II: *Blepharis ciliaris*-type, Pollen type-III: *Hygrophila polysperma* – type, Pollen type-IV: *Justicia adhatoda*-type, Pollen type-V: *Lepidagathis incurva*-type, Pollen type-VI: *Peristrophe paniculata*-type, Pollen type-VII: *Ruellia patula*-type and Pollen type-VIII: *Strobilanthes atropurpureus* – type. Within the family pollen diversity is significant enough for delimiting the tribes, subtribes and genera.

Introduction

Acanthaceae is a large family of nearly 250 genera and 2500 species (Mabberly, 1987). In Pakistan it is represented by 18 genera and 60 specific and infraspecific taxa; of which 44 are native (Malik & Ghafoor 1988). The family is characterized by having usually zygomorphic, persistent, 4-5-lobed. calyx, gamopetalous corolla, tube cylindrical or ventricose, the limb of 5, subequally spreading lobes or strongly 2-lipped. Stamens 4 and didynamous or 2, epipetalous. Ovary conical or oblong-cylindric, bicarpellate, syncarpous, superior, placentation axile. Fruits bivalved, 2-loculed capsule rarely drupaceous.

Scotland & Vollesen (2000) divided the family Acanthaceae into four subfamilies viz., 1. Nelsonioideae, 2. Acanthoideae (Acantheae), Ruellieae, Justicieae, Nemacanthus, Whitfieldieae, Andrographidae, Barlerieae) 3. Thunbergioideae, and 4. Avicennioideae.

Pollen morphological studies of the family Acanthaceae were conducted from time to time. The first palynological study was that of Radlkofer (1883), who investigated few taxa. However, Lindau (1893, 1895), carried out detailed study and recognized for the first time 11 pollen types within the family Acanthaceae. Since then number of workers studied the pollen of various taxa belonging to Acanthaceae (Natarajan (1957, Raj 1961; Huard 1962; Fageri & Iverson. 1964; Moore & Webb 1978; Scotland, 1992; Furness, 1990, 1994; Furness & Grant (1996), Mc Dade & Moody (1999). Mc Dade *et al.*, (2000) utilized palynological data in establishing the phylogenetic relationships of the tribe Justicieae. There are no reports on the pollen morphology of the family Acanthaceae from Pakistan with the exception of Qaiser & Perveen (1997) who examined the pollen of few species. The pollen morphology of Acanthaceae from Pakistan has not been investigated at length. The present investigations are the first comprehensive studies and the pollen of 75% native species of Pakistan have been examined with the help of light and Scanning Electron Microscope (SEM).

Materials and Methods

Pollen samples were obtained from Karachi University Herbarium (KUH) or the fresh material was collected from the field (The list of voucher specimen was deposited in the KUH. The pollen slides were prepared for light (LM) and Scanning Electron Microscopy (SEM) by the standard methods, described by Erdtman (1952).

For light microscopy: The pollen were mounted in unstained glycerine jelly and observations were made with a Nikon Type-2 microscope under (E40, 0.65) and oil immersion (E100, 1.25), using 10x eye piece. For each taxon 15-20 readings were taken and the following parameters were recorded: Polar length, Equatorial diameter, Colpus length, Mesocolpium, Apocolpium, Exine thickness, Ora diameter (in case of compound apertures).

For Scanning Electron microscopy: Pollen were suspended in a drop of water, directly transferred with a fine pipette to a metallic stub using double sided cellotape and coated with gold in a sputtering chamber (Ion sputter JFC-1100). Coating was restricted to 150A. The S.E.M examination was carried out on a Jeol Scanning Electron microscope JSM-T200 and JSM-6380A.

The terminology used is in accordance with Erdtman (1952), Kremp (1965), Faegri & Iversen (1964) and Walker & Doyle (1975).

Results and observations: General pollen morphology of the family Acanthaceae

Pollen usually radially symmetrical, isopolar rarely apolar, oblate-spheroidal to prolate or sub-oblate, porate, colpate, colporate and heterocolpate, amb trilobed or triangular, with apertures on the angles of the outline of the grain in polar view, often planaperturate, colpal membrane generally scabrate or granulated, rarely baculate, ora circular to transversely elliptic, sexine thicker or thinner than nexine, some times thinner at the polar region than at the equator. Tectum reticulate, reticulum of variable types from medium to coarse reticulate, or often lopho-reticulate, lumina perforated to baculate or scabrate. Eight pollen types are recognized on the basis of aperture, tectum and colpal membrane. Viz., Pollen type-I: *Barleria cristata*-type, Pollen type-II: *Blepharis ciliaris*-type, Pollen type-III: *Hygrophila polysperma*-type, Pollen type-IV: *Justicia adhatoda*-type, Pollen type-V: *Lepidagathis incurva*-type, Pollen type-VI: *Peristrophe paniculata*-type, Pollen type-VI: *Ruellia patula*- type and Pollen type-VIII: *Strobilanthes atropurpureus*-type.

Key to the pollen types

1. + Pollen homoaperturate.....2
 - Pollen heteroaperturate.....5
2. + Pollen porate..... Pollen type-VII: *Ruellia patula* - type
 - Pollen colpate or colporate.....3
3. + Pollen colpate.....Pollen type-II: *Blepharis ciliaris*-type
 - Pollen colporate.....4

- 4. + Tectum lopho reticulate.....Pollen type-I:*Barleria cristata*-type
 -Tectum coarsely reticulate..... Pollen type-V: *Lepidagathis incurva*-type
- 5. + Pollen 9- heterocolpate 3colpi with ora, 6colpi without ora.....7
 - Pollen 12- heterocolpate 4colpi with ora, 8 colpi without ora.....6
- 6.+ Tectum coarsely reticulate with regular pattern of muri
Polle type-VIII: *Strobilanthes atropurpureus*-type
 - Tectum coarsely reticulate –striate with irregular pattern of muri.....
Pollen type-III: *Hygrophila polysperma*
- 7. + Colpal membrane finely reticulate.....Pollen type-IV:*Justicia adhatoda*-type
 - Colpal membrane sub-psilatePollen type-VI: *Peristrophe paniculata*-type

Pollen type-I: *Barleria cristata*-type (Fig.1 A-F)

Pollen class: Tricolporate

P/E ratio:0.92

Shape: Oblate-spheroidal

Apertures: Colpus long sunken with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation:. Lophoreticulate

Measurements: Size: Polar axis P(82.50-) 84.53 ± 0.18 (-87.95) µm, C.V. 13.05, and equatorial diameter E(92.34-) 94.34 ± 0.82 (-96.93) µm, C.V. 4.01Tricolporate, colpi (28.72-) 31.71 ± 1.21 (-35.91) µm long, C.V. 13.01, colpal membrane granulated, ora distinct, ± circular, (8.97-) 13.21 ± 0.82 (-14.36) µm, C.V. 17.61. Mesocolpium (53.80-) 59.90 ± 1.93 (-68.21) µm, C.V. 10.19. Apocolpium (21.54-) 22.97 ± 0.87 (-25.13) µm, C.V. 8.55. Exine (17.95-)22.87 ± 0.87 (-23.33) µm thick, C.V. 12.61, sexine baculate, thicker than nexine. Tectum lopho-reticulate, lophate with irregular mural pattern, muri fused, luminae ± scabrate or baculate.

Species included: *Barleria prionitis* L., *Barleria cristata* L., and *Barleria hochstetteri* Nees.

Key to the species

- + Exine 17.95-23.33 µm thick, lumina scabrate, with fused muri.....*Barleria prionitis*
- Exine 8.97-10.77 µm thick, luminabaculate, with free or fused muri.....2
- + Polar length 53-62 um.....*Barleria hochstetteri*
- Polar length 81-83 um.....*Barleria cristata*

Table. I. Pollen Characters of different species of pollen type I- *Barleria cristata*

Name of Taxa	Polar length in µm	Equatorial diameter in µm	Colpus length in µm	Exine Thickness in µm
<i>Barleria cristata</i> L.	83.85 (83.88± 1.44) 83.11	82.49 (83.37± 2.21) 84.21	33.31(34.46± 0.32)	9.99 (11.59± 0.12) 10.77
<i>Barleria prionitis</i> L.	82.50 (86.58± 0.18)	92.34 (93.3± 0.82) 96.93	28.72(31.71± 1.21) 35.91	17.95(21.87± 0.87) 23.33
<i>Barleria hochstetteri</i> Nees	53.85 (57.88± 1.84) 62.82	57.49(62.37± 2.21) 68.21	32.31(34.46± 6.37) 35.91	8.97 (10.59± 0.18) 10.77

Pollen type-II: *Blepharis ciliaris*-type (Fig.2 A&B)**Pollen class:** Tricolpate**P/E ratio:** 1.46.**Shape:** Prolate**Aperture:** Colpus long narrow with acute ends.**Exine:** Sexine thicker than nexine.**Ornamentation:** Reticulate.

Measurements: Size: Polar axis P(22.21-) 23.73 ± 0.15 (-23.82) μm , and equatorial diameter E (15.95-) 16.51 ± 1.22 (-17.13) μm . trilobed, with apertures on the angles of the outline of the grain in polar view, colpi (14.13-) 14.25 ± 1.21 (-15.31) μm long, intruding. Mesocolpium C. 13.3 μm . Apocolpium C. 3.23 μm . Exine (2.52-) 2.51 ± 1.23 (-4.59) μm thick, sexine homobaculate, distinctly thinner than nexine. Tectum reticulate.

Species included: *Blepharis ciliaris* (L.) B.L. Burtt, *Blepharis maderaspatensis* (L.) Roth *Blepharis sindica* Stocks ex T. Anders.

Note: The pollen of various species in this type are indistinguishable with the exception of the size. *Blepharis ciliaris* has the smallest pollen than the remaining two species.

Table II. Pollen Characters of different species of pollen type II- *Blepharis ciliaris*.

Name of Taxa	Polar length in μm	Equatorial diameter in μm	Colpus length in μm	Exine Thickness in μm
<i>Blepharis ciliaris</i> (L.)	22.20 (23.11 \pm 0.51)	15.95 (16.50 \pm 1.22)	14.13 (14.25 \pm 1.21)	2.51 (2.51 \pm 1.32)
B.L. Burtt	23.82	17.13	15.31	4.95
<i>Blepharis maderaspatensis</i> (L.) Roth	43.91 (44.05 \pm 0.54)	22.95 (23.13 \pm 1.62)	35.13 (35.25 \pm 0.84)	2.51 (6.31 \pm 0.22)
<i>Blepharis sindica</i> Stocks ex T. Anders.	45.71 (38.23 \pm 0.31)	25.13 (22.10 \pm 0.62)	36.31 (27.25 \pm 0.71)	3.49 (21.21 \pm 0.23)
	48.47	25.13	32.31	3.49

Pollen type- III: *Hygrophila polysperma*-type (Fig.2 C-F, Fig3. A & B)**Pollen class:** Heterocolpate**P/E ratio:** 0.81-1.86**Shape:** Prolate, or sub-oblate or prolate-spheroidal**Apertures:** Colpus long sunken with acute ends.**Exine:** Sexine thicker thinner than nexine.**Ornamentation:** Tectum coarsely reticulate.

Measurements: Size: Polar axis P(39.49-) 45.03 ± 0.96 (-50.26) μm , C.V. 7.99, and equatorial diameter E (46.65-) 55.04 ± 1.38 (-64.62) μm , C.V. 9.67. Sub-oblate, 20-23 heterocolpate (4 colporate, 16-18 colpate), 20-22 lobed, colpi (32.31-) 36.61 ± 0.85 (-39.49) μm in length C.V. 7.38, tapering at both the ends, colpal membrane sparsely granulated, ora distinct, transversely elliptic, (8.79-) 10.17 ± 0.37 (-11.77) μm in diameter, C.V.9.04. Mesocolpium (4.66-) 5.22 ± 0.87 (-5.38) μm , C.V. 4.98, Apocolpium (3.59-) 4.37 ± 0.49 (-7.51) μm , C.V. 35.91. Exine (3.23-) 3.41 ± 0.05 (-3.59) μm , C.V. 5.27, sexine thinner than nexine. Tectum coarsely reticulate-striate, consists of primary network of coarse tectal ridges (muri), surrounding spaces (lumina) containing few to many smaller or large perforations, perforation per luminae usually varying with the size of reticulum.

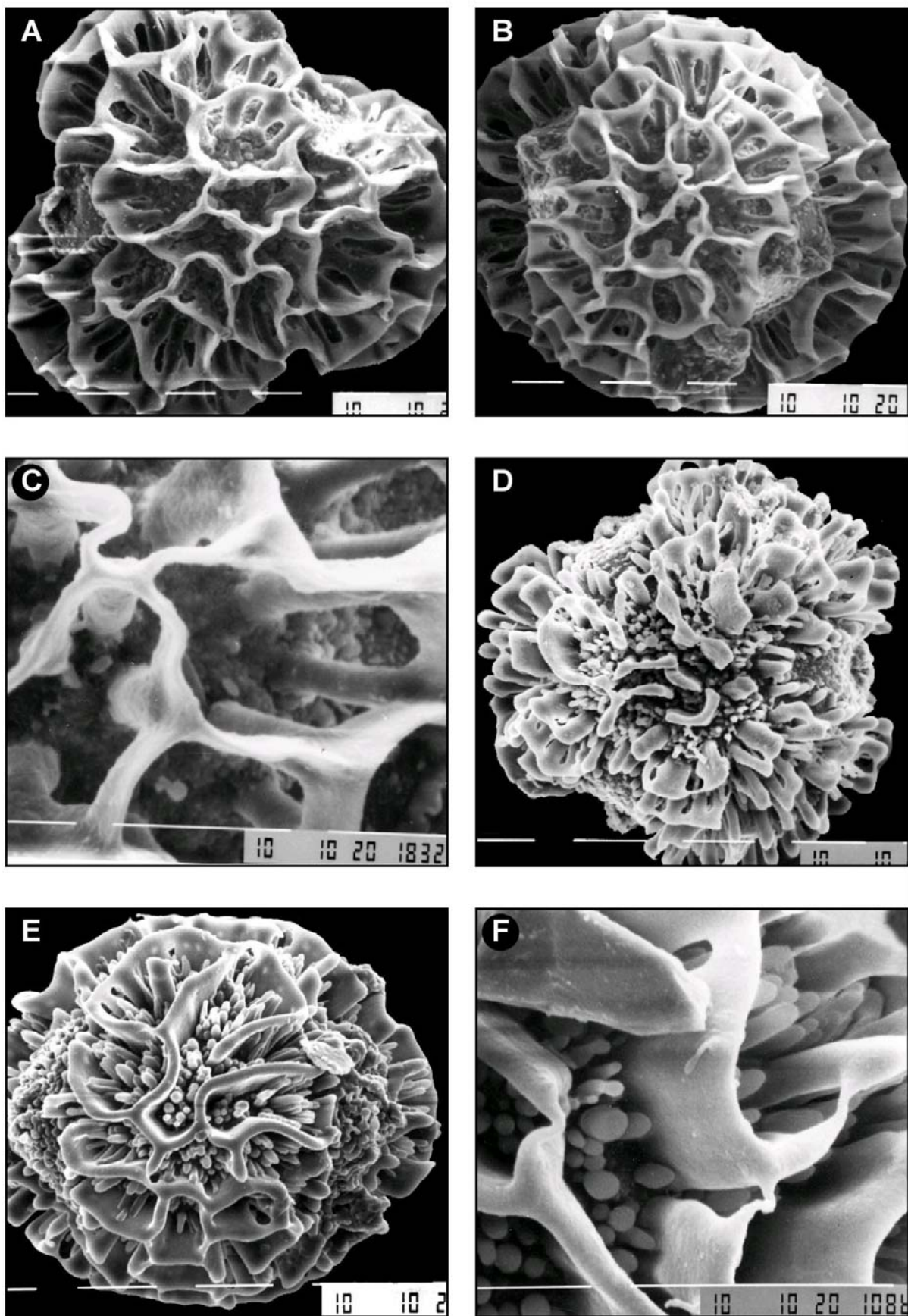


Fig. 1. Scanning Electron micrographs of pollen grains: *Barleria hochstetteri*: A, Polar view, B, Equatorial view, C, Exine pattern. *Barleria parionitis*: D, Polar view; E, Equatorial view; F, Exine pattern. (Scale bar: A-F=10 μ m).

Species included: *Aechmanthera tomentosa* (Wall.) Nees, *Hygrophila polysperma* (Roxb.) T. Anders., *Hygrophila quadrivalvis* (Buch.-Ham.) Nees

Key to the species

1. + Pollen prolate -spheroidal.....2
 - Pollen sub-oblate.....*Hygrophila polysperma*
2. + Pollen prolate-spheroidal*Aechmanthera tomentosa*
 - Pollen prolate.....*Hygrophila quadrivalvis*

Table. III. Pollen Characters of different species of pollen type III- *Hygrophila polysperma*

Name of Taxa	Shape	Polar length in μm	Equatorial diameter in μm	Colpus length in μm	Exine thickness in μm
<i>Hygrophila polysperma</i> (Roxb.) Anders.	Sub-oblate	39.49	46.65	32.31	3.23
		(45.03 \pm 0.96)	(55.04 \pm 1.38)	(36.61 \pm 0.85)	(3.41 \pm 0.05)
<i>Hygrophila quadrivalvis</i> (Buch-Ham) Nees	Prolate Spheroidal	50.26	64.62	39.49	3.59
		41.49	39.65	32.31	3.23
		(43.03 \pm 0.26)	(42.04 \pm 1.36)	(41.61 \pm 0.25)	(3.41 \pm 0.9)
<i>Aechmanthera tomentosa</i> (Wall.) Nees	Prolate	45.56	45.62	39.49	3.59
		52.49	31.65	46.31	2.3
		(56.03 \pm 0.16)	(36.04 \pm 1.30)	(50.61 \pm 0.84)	(3.111 \pm 1.05)
		60.26	41.62	54.49	3.59

Pollen type-IV: *Justicia adhatoda*-type (Fig. 3 C & D)

Pollen class: Heterocolpate

P/E ratio: 1.50

Shape: Prolate to per-prolate

Apertures: Colpus long sunken with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation: Reticulate

Measurements: Size: Polar axis P(38.49-) 39.03 \pm 1.5 6 (40.26) μm , and equatorial diameter E(25.65-) 26.04 \pm 1.28 (-27.62) μm . Prolate to per-prolate, 20-23 heterocolpate (3 colporate, 12-18 colpate), 20-22 lobed, colpi (32.21-) 36.11 \pm 0.25 (-39.39) μm in length, tapering at both the ends, colpal membrane finely reticulate, granulated, ora distinct, transversely elliptic, (8.79-) 10.17 \pm 0.34 (-11.77) μm in diameter. Mesocolpium (4.46-) 4.99 \pm 0.87 (-5.38) μm . Apocolpium (3.59-) 4.37 \pm 0.41 (-5.51) μm . Exine (3.23-) 5.21 \pm 1.05 (-5.19) μm , sexine thinner than nexine. Tectum finely reticulate.

Species included: *Justicia adhatoda* L. *J. brandegeana* Wassh. & Smith, *J. gendarussa* Burm.f., *J. japonica* Thunb., *J. peploides* (Nees) T. Anders. , *J. vahlii* Roth, and *J. tinctoria* (Oerst.) D.Gibson.

Key to the species

- 1.+ Pollen prolate..... group-1 (*Justicia adhatoda*, *J. gendarussa* ,
J. japonica, *J. brandegeana* and *J. vahlii*).
 - Pollen Per-prolate.....group-II(*Justicia tinctoria* and *J. peploides*)

Table. IV. Pollen Characters different species pollen type-IV- *Justicia adhatoda*

Name of Taxa	Shape	Polar length in μm	Equatorial diameter in μm	Colpus length in μm	Exine thickness in μm
<i>Justicia adhatoda</i> L.	Prolate	38.49(39.03 \pm 1.56) 40.26	25.65(26.04 \pm 1.28) 37.62	32.21 (36.11 \pm 0.25) 39.39	1.23(2.1 \pm 1.05) 5.19
<i>Justicia brandegeana</i> Wassh. & Smith	Prolate	41.49(44.83 \pm 0.16) 44.26	26.65 (26.04 \pm 1.22) 26.62	32.31 (30.61 \pm 0.15) 39.49	1.02(1.41 \pm 0.11) 3.59
<i>Justicia gendurussa</i> Burm. f.	Prolate	30.29 (31.22 \pm 0.17)31.46	23.65 (23.04 \pm 1.32) 24.62	26.31 (27.61 \pm 0.55) 28.19	c.1.52
<i>Justicia japonica</i> Thunb.	Prolate	26.30(28.03 \pm 1.56)30.24	15.78(17.00 \pm 1.28) 19.72	23.44 (24.11 \pm 0.25) 26.01	c.1.73
<i>Justicia peploides</i> (Nees) T.Anders.	Per- prolate	61.21(61.03 \pm 1.56) 62.80	28.93(29.50 \pm 1.28) 31.93	c.57.86	c.1.34
<i>Justicia tinctoria</i> (Oerst.) D. Gibson	Per- Prolate	57.29 (59.03 \pm 0.26) 62.26	26.65 (26.04 \pm 1.35) 27.62	34.31 (44.61 \pm 0.25) 54.49	c.1.32

Pollen type-V: *Lepidagathis incurva*-type (Fig.3 E & F)

Pollen class: Tricolporate

P/E ratio: 1.09-1.55

Shape: Prolate or prolate-spheroidal**Apertures:** Colpus long narrow**Exine:** Sexine thinner than nexine**Ornamentation:** Coarsely reticulate

Measurements:Size: Polar axis P(26.50-)29.53 \pm 0.22 (-31.95) μm , , and equatorial diameter E(18.34-) 18.94 \pm 0.82 (-19.93) μm . Prolate or prolate-spheroidal, tricolporate, colpi (24. 72-) 26.71 \pm 1.41 (-35.91) μm long, colpal membrane psilate, ora distinct, \pm circular, (8.97-) 11.66 \pm 0.62 (-14.36) μm , Mesocolpium (53.80-) 60. 50 \pm 1.5 3 (-68.21) μm . Apocolpium (21.54-) 23.11 \pm 0.43 (-25.13) μm , Exine (17.95-) 20.64 \pm 0.23 (-23.33) μm thick, sexine thicker than nexine. Tectum coarsely reticulate, lumina variable in size, larger towards colpi, luminae \pm granulated.

Species included: *Eranthemum pulchellum*, Andrews, *Lepidagathis lutea* Dalz., *Lepidagathis trinervis* Nees and *Lepidagathis incurva* Buch.-Ham. ex D. Don.

Key to the species

- 1.+ Pollen Prolate-spheroidal.....*Eranthemum pulchellum*
 - Pollen prolate.....2
2. + Polar length 26-34.95 μm *Lepidagathis lutea*, *L. incurva*
 - Polar length 62-64 μm *Lepidagathis trinervis*

Table. V. Pollen characters different species of pollen typeV- *Lepidagathis incurva*.

Name of Taxa	Shape	Polar length of pollen in μm	Equatorial diameter in μm	Colpus length in μm	Exine thickness in μm
<i>Eranthemum pulchellum</i> Andrews.	Prolate-spheroidal	58.50 (59.53 \pm 0.88)	52.34 (57.34 \pm 0.8)	28.72 (37.71 \pm 1.11)	1.7 (2.1.89 \pm 1.82)
<i>Lepidagathis lutea</i> Dalz.		26.50 (28.53 \pm 0.22)	18.34 (18.94 \pm 0.82)	211.72 (26.71 \pm 1.41)	17.95 (2.1 \pm 0.23)
<i>Lepidagathis trinervis</i> Nees	Prolate	31.95 (63.53 \pm 0.18)	19.93 (39.54 \pm 0.82)	35.91 (30.71) \pm 1.65	2.3 (2.0 \pm 0.87)
<i>Lepidagathis incurva</i> Buch.-Ham. ex D. Don.		62.50 (33.93 \pm 1.21)	39.34 (23.11 \pm)	25.72 c. 31.56	1.7.95 c. 2.8
		64.95 34.19	40.93 23.67		2.33

Pollen type-VI: *Peristrophe paniculata* – type (Fig.4 A & B)

Pollen class: Heterocolpate

PE ratio: 1.38

Shape: Prolate

Apertures: Colpus long narrow

Exine: Sexine thinner at the polar regions

Ornamentation: Reticulate

Measurements:Size: Polar axis P(35.91-)40.40 \pm 0.89 (-46.67) μm , C.V. 8.16, and equatorial diameter E(28.72-) 31.63 \pm 0.53 (-35.91) μm , C.V. 6.81 Prolate, 9-heterocolpate (3-colporate, 6 colpate), triangular, planaperturate, colpi (35.91-) 37.44 \pm 0.53 (-39.49) μm long, C.V. 4.37, colpal membrane minute granulated, ora distinct, longitudinal, \pm circular, (3.59-) 4.30 \pm 0.27 (-5.38) μm in diameter, C.V. 19.41. Mesocolpium (25.13-)26.69 \pm 0.71 (-28.72) μm , C.V. 5.79. Apocolpium C.3.59 μm . Exine (3.59-) 5.20 \pm 0.37 (-6.82) μm thick, C.V. 15.61, sexine homobaculate, undulate, thinner at the pole than at the equator. Tectum coarsely reticulate, muri with elevated ends, lumina \pm pentagonal, scabrate, 0.91-1.26 in diameter.

Species included: *Peristrophe paniculata* (Forssk.) Brummitt, *Dicliptera bupleuroides* Nees and *Lepidagathis incurva* Buch.-Ham.ex D.Don

Key to the species

- + Polar length of pollen grain 56-58 μm*Dicliptera bupleuroides*
- polar length of pollen grains 35-45.7 μm*Peristrophe paniculata*

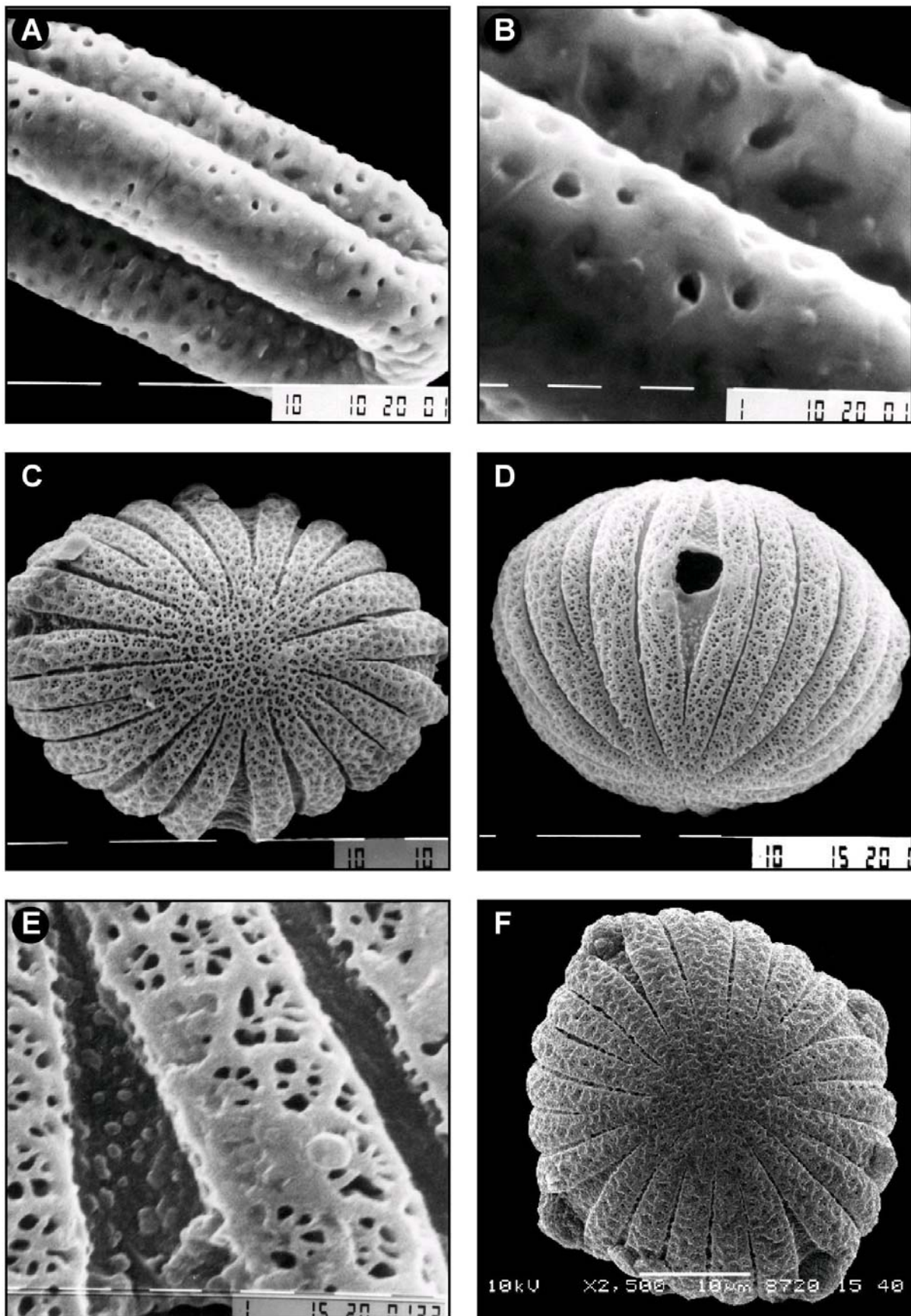


Fig. 2. Scanning Electron micrographs of pollen grains: *Blepharis indica*: A, Equatorial view B, Exine pattern; *Hygrpphila polysperma*: C, Polar view; D, Equatorial view; E, Exine pattern. *Hygrophila quadrivalvis*: F, Polar view. (Scale bar: A,C,D & F= 10 μ m; B & E= 1 μ m)

Table: VI Pollen characters different species of pollen type VI- *Peristrophe paniculata*

Name of Taxa	Polar length in μm	Equatorial diameter in μm	Colpus length in μm	Exine Thickness in μm
<i>Dicliptera</i>	56.91	31.12	40.91	3.51
<i>bupleuroides</i>	(57.40 \pm 0.91)	(32.620 \pm 1.22)	(42.44 \pm 1.21)	(6.31 \pm 1.32)
Nees	58.10	35.91	44.49	6.82
<i>Peristrophe</i>	35.91	28.72	35.91	3.59
<i>paniculata</i>	(42.40 \pm 0.89)	(30.63 \pm 0.37)	(38.44 \pm 0.53)	(6.31 \pm 0.37)
(Frossk.)	45.71	35.91	39.49	3.49
Brummit.				

Pollen type-VII: *Ruellia patula* – type (Fig.4 C-F)**Pollen Class:** Porate**P/ratio:** 1.00**Shape:** Spheroidal**Apertures:** more or less circular**Exine:** thicker than nexine.**Ornamentation:** Very coarsely reticulate.**Measurements: Size:** (86.16-) 95.74 \pm 0.93 (-100.51) μm in diameter.

Spheroidal, 5-7 porate, \pm pore circular, C.7.51 μm in diameter. Exine (4.30-) 4.93 \pm 0.21 (-5.56) μm thick, sexine thicker than nexine. Tectum very coarsely reticulate with densely granulated lumina, luminae \pm polygonal in shape 7-8.5 μm in diameter.

Species included: *Ruellia patula* Jacq. , *R.brittoniana* E. Leonard , *R.prostrata* Poir. *R. linearibracteolata* Lindau, *R. tuberosa* L.

Table. VII. Pollen Characters of different species of pollen type VII- *Ruellia patula*

Name of Taxa	Pollen diameter in μm	Pore diameter in μm	Exine thickness in μm
<i>Ruellia tuberosa</i> L.	73.16 (72.74 \pm 0.73) 73.61	c.6.51	4.31(5.45 \pm 0.11) 5.66
<i>Ruellia patula</i> Jacq.	86.16 (91.74 \pm 0.93) 100.5	c. 7.51	4.30(5.45 \pm 0.21) 5.56
<i>Ruellia brittoniana</i> Leonard	53.66 (54.74 \pm 0.93) 54.41	c. 2.51	3.30(3.61 \pm 0.31) 5.55
<i>Ruellia prostrata</i> Poir.	58.16(59.80 \pm 0.93) 59.51	c. 7.51	3.59 (5.45 \pm 0.11) 5.55
<i>Ruellia linearibracteolata</i> Lindau	36.16(51.74 \pm 0.23) 52.51	c. 4.41	4.30(5.45 \pm 0.21)5.61

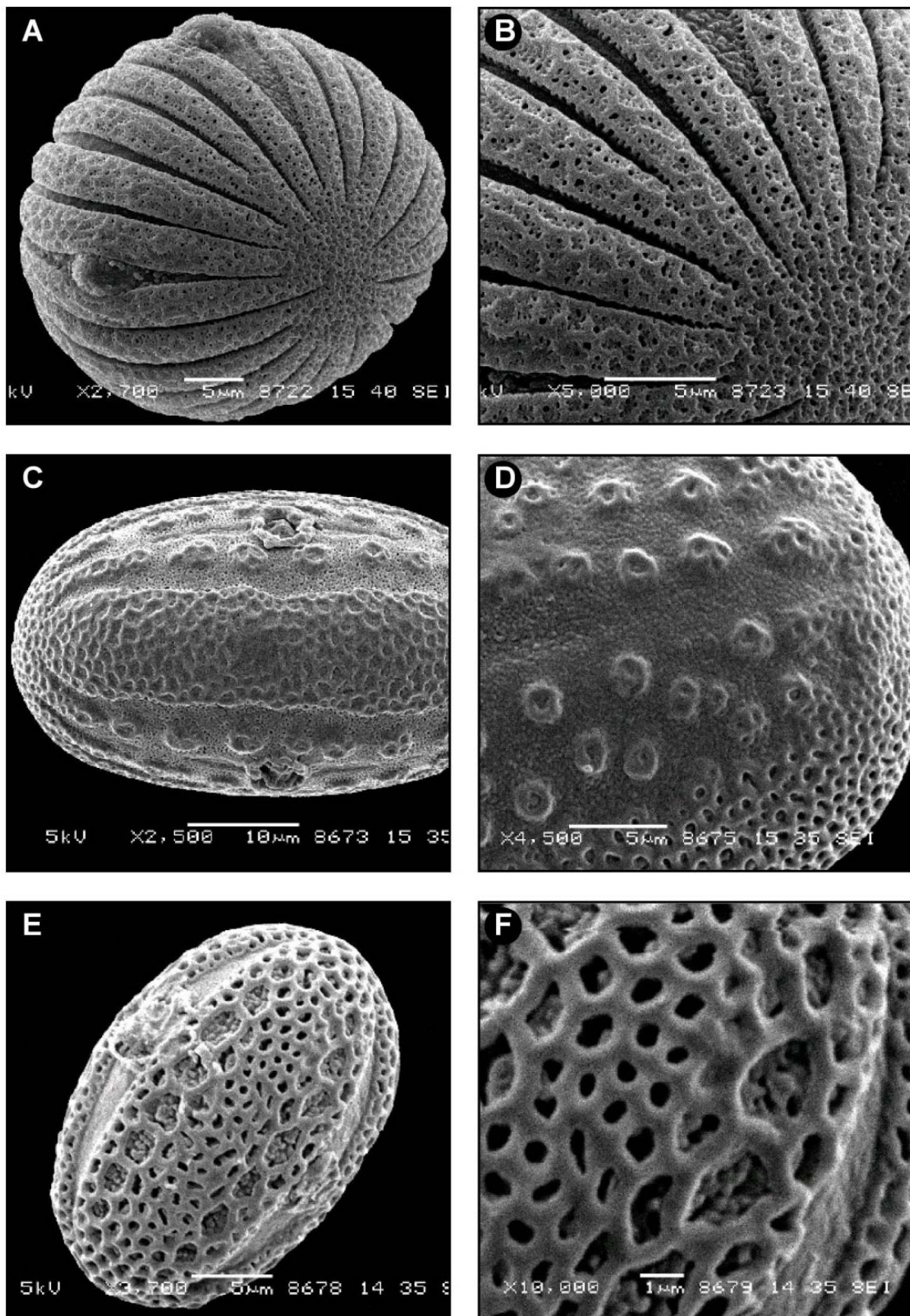


Fig. 3. Scanning Electron micrographs of pollen grains: *Hygrophila quadrivalvis*: A, Equatorial view, B, Exine pattern. *Justicia Japonica*: C, Equatorial view; D, Exine pattern; *Lepidagathis incurve*: E, Equatorial view; F, Exine pattern. (Scale bar: A, B, D & E= 5 μ m; F=1 μ m; C= 10 μ m).

Key to the species

- 1 + Pollen 36-52 μm in diameter*Ruellia linearibracteolata*
 - Pollen 53-100 μm in diameter.....2
- 2 + Pollen 86-100 μm in diameter*Ruellia patula*
 - pollen less than 83 μm in diameter..... *Ruellia tuberosa*, *Ruellia brittoniana*, *Ruellia prostrata*

Pollen type-VIII: *Strobilanthes atropurpureus* –type (Fig5. A-F)

Pollen class: heterocolpate

P/E ratio: 1.6

Shape: Prolate

Aperture: long

Exine: thinner at the polar regions.

Ornamentation reticulate

Measurements: Size: Polar axis P(57.91-) 66.20 \pm 0.22 (-75.67) μm , and equatorial diameter E(39.72-) 41.13 \pm 1.63 (-44.91) μm .

Prolate, 12-heterocolpate (4-colporate, 8 colpate), triangular, planaperturate, colpi (35.91-) 37.44 \pm 0.54 (-39.49) μm long, colpal membrane minute granulated, ora distinct, lo-longate, \pm circular, (3.59-) 4.40 \pm 0.26 (-5.38) μm in diameter. Mesocolpium (25.13-) 26.69 \pm 0.11 (-28.72) μm , Apocolpium C.3.59 μm . Exine (3.22-) 4.61 \pm 0.32 (-6.00) μm thick, sexine homobaculate, undulate, thinner at the pole than at the equator. Tectum coarsely reticulate lumina, finely reticulate.

Species included: *Strobilanthes atropurpureus* Nees, *Strobilanthes dalhousieanus* (Nees) Clarke and *Strobilanthes urticifolia* Wall. ex Kuntze

Key to the species

1. + Polar length 42-46 μm *Strobilanthes urticifolia*
 - Polar length 57-75 μm 2
2. + Colpi 35-39 μm *Strobilanthes atropurpureus*
 - Colpi 59-70 μm *Strobilanthes dalhousieanus*

**Table VIII. Pollen characters of different species of pollen type VIII-
*Strobilanthes atropurpureus***

Name of Taxa	Polar length in μm	Equatorial diameter in μm	Colpus length in μm	Exine Thickness in μm
<i>Strobilanthes atropurpureus</i> Nees	35.91 (38.44 \pm 0.54)	39.72 (41.13 \pm 1.63)	35.91 (38.44 \pm 0.54)	3.22 (6.31 \pm 0.32)
<i>Strobilanthes dalhousieanus</i> (Nees) Clarke	39.49 (62.91 (67.90 \pm 0.89)	44.91 (28.12 (26.63 \pm 0.53)	39.94 (59.541 (64.44 \pm 0.23)	6.00 (3.59 (6.21 \pm 0.37)
<i>Strobilanthes urticifolia</i> Wall. ex Kuntze	72.67 (23.6 \pm 0.51)	35.3 (19.61 \pm 0.49)	70.49 (38.44 \pm 0.53)	6.22 (6.21 \pm 0.29)
	25.91	23.81	39/49	6.82

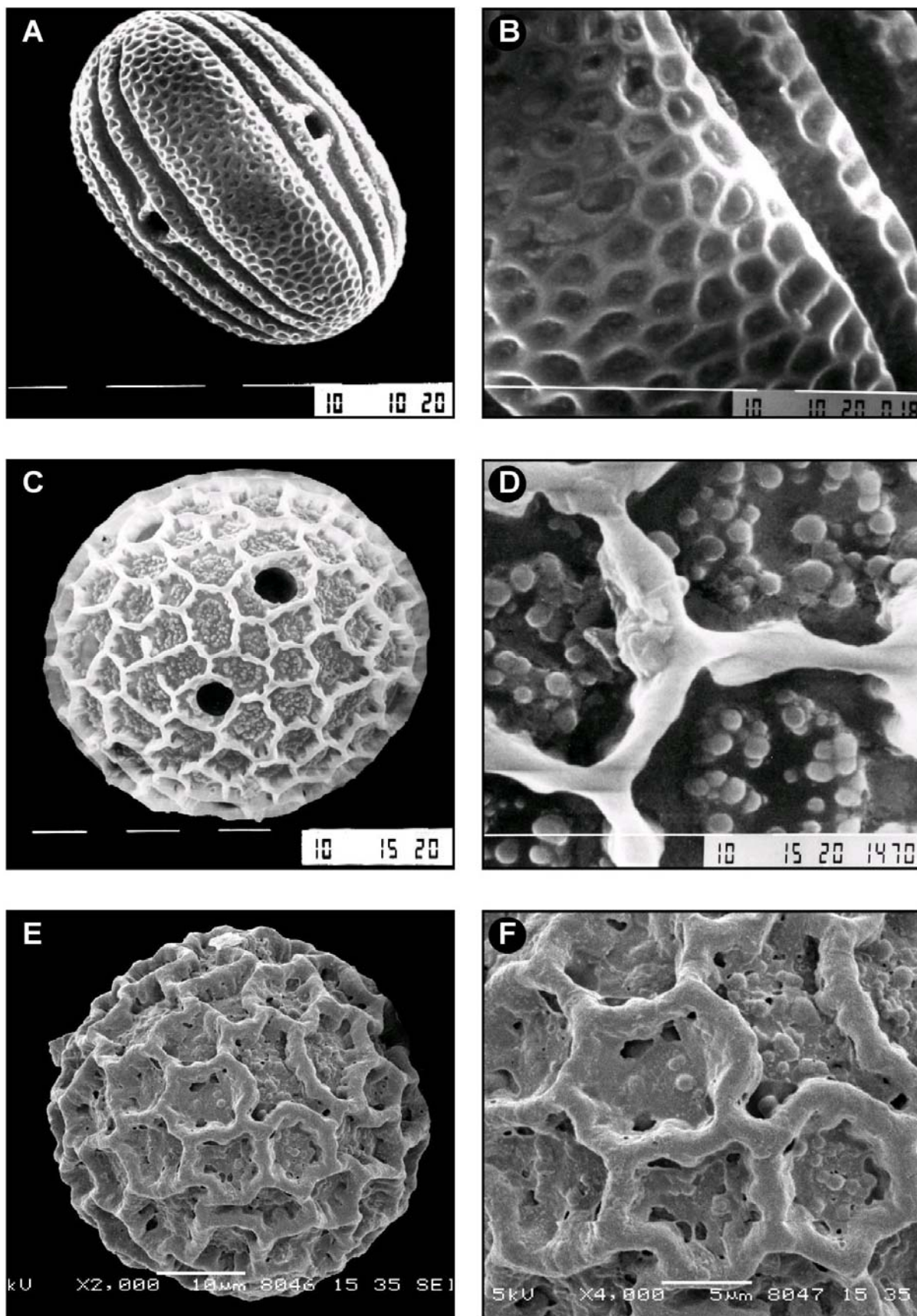


Fig. 4. Scanning Electron micrographs of pollen grains: *Peristrophe Paniculata*: A, Equatorial view; B, Exine pattern *Ruellia patula*: C, Pollen grain; D, Exine pattern. *Ruellia Prostrata* E, Pollen grain; F, Exine pattern. (Scale bar: A-E= 10 μ m ; F= 5 μ m).

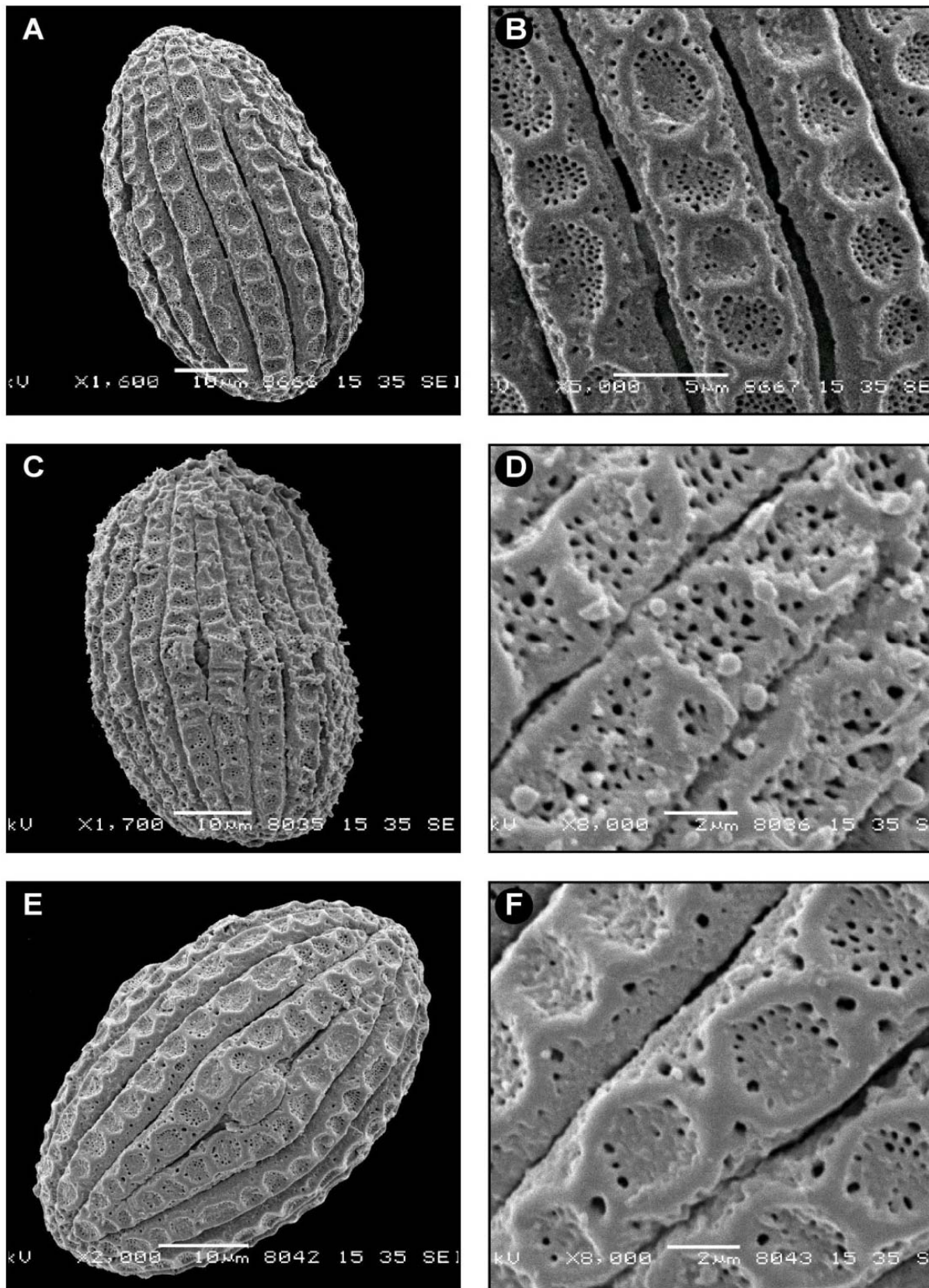


Fig. 5. Scanning Electron micrographs of pollen grains: *Strobilanthes atropurpureus*: A, Equatorial view; B, Exine pattern. *Strobilanthes dalhusieanus*: C, Equatorial view; D, Exine pattern. *Strobilanthes urticifolia*: E, Equatorial view; F, Exine pattern. (Scale bar: A, C & E=10 μm; B=5 μm; D & F=2 μm).

Discussion

Acanthaceae is a eurypalynous family. There is a remarkable diversity in the pollen morphology in various characters such as pollen size, shape, aperture (colpi or pore, simple or compound) and tectum. This variation has already been observed by the previous workers, such as, Radlkofer (1883) and Lindau (1893, 1895), He recognized 11 pollen types, which were used in subfamilial classification. Similarly, Rizzini (1947) examined pollen morphology of many Brazilian genera and utilized pollen characters for delimiting the genera. Muller et al. (1989) recognized 26 different pollen types within the family Acanthaceae from Madagascar and several Islands of Indian Ocean. Daniel (1998) also observed diversity in pollen morphology within the family Acanthaceae from Mexico. He was of the opinion that the diversity was taxonomic rather than geographic nature as the diversity of Acanthaceous pollen in various parts of the world was almost equal or even more.

Present pollen study is based on 30 species of Acanthaceae, distributed in 11 genera i.e., *Aechmanthera*, *Barleria*, *Blepharis*, *Dicliptera*, *Eranthemum*, *Hygrophila*, *Justicia*, *Lepidagathis*, *Peristrophe*, *Ruellia* and *Strobilanthes*. Our findings are in agreement with the previous workers as tremendous palynological diversity has been also observed in all the taxa studied. Shape class varies from prolate to subprolate or oblate-prolate – spheroidal rarely per - prolate. Apertural type (number, position, size and shape) varies from heterocolpate pollen, which is a dominant type, followed by tricolporate, porate and tricolpate. Similarly, tectum also exhibits considerable variation from fine-coarse reticulate, reticulate-rugulate, lophoreticulate to rarely foveolate.

On the basis of apertural type, shape class and exine sculpturing the family Acanthaceae has been divided into eight distinct pollen types viz., Pollen type-I: *Barleria cristata*, Pollen type-II: *Blepharis ciliaris*-type, Pollen type-III: *Hygrophila polysperma* – type, Pollen type-IV: *Justicia adhatoda*-type, Pollen type-V: *Lepidagathis incurva*-type, Pollen type-VI: *Peristrophe paniculata*-type, Pollen type-VII: *Ruellia patula* - type and Pollen type-VIII: *Strobilanthes atropurpureus* – type.

Pollen type-I: *Barleria cristata*-type is easily recognized by its unique type of tectum i.e., lophoreticulate with tricolporate aperture. Balkwill and Balkwill (1998) also reported coarsely reticulate interaperturate, lophoreticulate tectum in other species of *Barleria*. Pollen type-II: *Blepharis ciliaris*-type is characterized by its colpate pollen. Erdtman (1952) also reported similar pollen within this genus. Pollen type-III: *Hygrophila polysperma*-type, usually recognized by its heterocolpate pollen (colpi alternating with pseudo colpi or colpi with in the same pollen). Two genera viz. *Aechmanthera* and *Hygrophila* fall in this pollen type. Pollen data suggest close relationship of both the genera. Pollen type-IV: *Justicia adhatoda* -type representing single genus (*Justicia*), is easily delimited by having heterocolpate pollen with reticulate margin, reticulum is arranged in circular pattern. This pollen type is fairly homogenous as it accommodates all the seven species of the genus *Justicia*. This type is characterized by heterocolpate pollen which is further divided in two groups on the basis prolate and prolate pollen. The data from molecular sequences, morphology and cytology indicate that the tribe Justiceae are monophyletic (McDade *et al.*, 2000).

In the pollen type-V: *Lepidagathis incurva*-type, pollen are tricolporate with distinct size of reticulum at polar and mesocolpial regions (Fig. 3). Within this pollen type two genera are included viz., *Eranthemum* and *Lepidagathis*, both are easily delimited on the shape class as in the genus *Eranthemum* pollen are prolate-spheroidal, whereas *Lepidagathis* has prolate pollen.

Pollen type VI: *Peristrophe paniculata*-type accommodates two genera *Dicliptera* and *Peristrophe* both have heterocolpate pollen similar to pollen type - V (*Justicia adhatoda*-type) but differs in having simple reticulate or subpsilate colp margin. However, these genera are further separated on the basis of shape (see key to the pollen types).

Pollen type-VII: *Ruellia patula*-type has porate pollen with coarsely reticulate tectum, 5 taxa fall in this pollen type which are easily separated on the basis of pollen diameter (see key to the pollen types). Raj (1961) also observed similar type of pollen grains in the genus *Ruellia*. Our observations are also in agreement with the finding of Tripp (2007) who demonstrated the monophyly of the genus *Ruellia* in the tribe Ruellieae on the basis of ribosomal ITS region and the chloroplast trnG-trnR.

Pollen type-VIII: *Strobilanthes atropurpureus*-type pollen has 4 colpi alternating with and 8-16 pseudo colpi similar to Pollen type -III (*Hygrophila polysperma* - type), *Strobilanthes atropurpureus*-type is recognized by having bireticulate tectum. Carine & Scotland (1998) reported much variation with the genus *Strobilanthes* and divided the genus into 22 pollen types on the basis of shape, apertural type and exine ornamentation. In the present study only three species of strobilanthes have been investigated. Pollen of all the three species are quite similar and differ only in the size and the length of the colpi.

Present Pollen morphological studies seem to be useful at the tribal, subtribal and generic level. All the Pakistani genera consists of the species that are relatively homogenous as far as the pollen characters are concerned. The species of not a single genus (11 genera studied) fall in two different pollen types. This homogeneity in various pollen characters has also been observed in Mexican genera by Daniel (1998) also.

The tribal classification of the family Acanthaceae is also well correlated with pollen morphology. Pollen type -I: *Barleria cristata* type. Pollen type III: *Hygrophila polysperma*-type, Pollen type VII: *Ruellia patula* type and Pollen type VIII: *Strobilanthes atropurpureus*-type are mostly found in the tribe Ruellieae where as pollen type-II: *Blepharis ciliaris* is restricted to the tribe Acanthaceae. Pollen type -V: *Lepidagathis incurva* type has representatives of two different genera belonging to two different tribes Justiceae and Lepidagathideae which show the close relationship of both the tribes.

Pollen of Acanthaceae are \pm similar to Boraginaceae, because both have (*Heliotropium* of Boraginaceae and *Peristrophe*, *Aechmanthera*, *Aphelandra*, *Hygrophila* and *Strobilanthes*) heterocolpate grains. However, the tectal surface of pollen of both the families are quite distinct. Some of the pollen types in Acanthaceae are \pm similar to Bignoniaceae and Pedaliaceae (Erdtman, 1952). In the family Bignoniaceae colpate grains are found with reticulate tectum, while in Acanthaceae (*Blepharis*) colpate grains with foveolate tectum are observed.

According to molecular studies (McDade *et al.*, 2005) family Acanthaceae s.l. are monophyletic. The present palynological data support this contention as the classification at the tribal level is well correlated with pollen morphology also.

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