

POLLEN FLORA OF PAKISTAN–LIV. RUBIACEAE

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Abstract

Pollen morphology of 50 species representing 20 genera of the family Rubiaceae from Pakistan has been examined by light and scanning electron microscope. Pollen grains usually radially symmetrical, isopolar, mostly prolate-spheroidal to sub-prolate, often oblate-spheroidal - sub-oblate rarely prolate. Aperture colpate to pantocolpate, or 3-10-colporate, sexine thicker or thinner than nexine. Tectal surface mostly spinulose or scabrate-punctate, reticulate or rugulate - reticulate often psilate. On the basis of apertural types and exine ornamentation, 9 distinct pollen types are recognized viz., *Argostemma sarmentosum*-type, *Aitchisonia rosea*-type *Galium elegans*-type, *Galium tenuissimum*-type, *Gaillonia macrantha*-type, *Jaubertia aucheri*-type, *Oldenlandia nudicaulis*-type, *Oldenlandia umbellata*-type and *Pseudogaillonia hymenostenphana*-type

Introduction

Rubiaceae is a large family of c. 450 genera, approximately 6500 species, largely of tropical and subtropical in distribution but some in temperate regions and few arctic in distribution (Mebberley, 1987). It is represented in Pakistan by 33 genera and c. 87 species (Nazimuddin & Qaiser, 1989).

Cronquist (1968) placed the family Rubiaceae in the subclass Asteridae within the order Rubiales. He considered Rubiales to be related to the Gentianales and Dipsacales (especially Caprifoliaceae). Chase *et al.*, (1993) also placed Rubiaceae among the families of Gentianales but not near to Dipsacales. Thorne (1968) and Takhtajan (1969) also treated this family under the order Rubiales.

The family is characterized by opposite and interpetiolar stipules or with whorled leaves without interpetiolar stipules, The corolla is regular, with isomerous stamens attached to the corolla tube and inferior ovary having two or more locules with axil placentation. Fruit capsule, berry or drupe. Chief genera of Rubiaceae are *Rubia*, *Hedyotis*, *Cinchona*, *Gardenia*, *Cephalanthus*, *Coffea*, *Galium*, *Pinckneya* and *Richardia*.

Pollen morphology of the family has been examined by Erdtman (1952, 1971), Huysmans *et al.*, (1994), Huysmans (1998), Kuprianova & Alyoshina (1978). Nowicke & Skvarla (1979), Persson (1993), Puff *et al.*, (1996), Vinckier *et al.*, (2000), Sotolongo Molina (2002) examined pollen morphology of 38 genera of the family Rubiaceae. There are no reports on pollen morphology of the family Rubiaceae from Pakistan. Present investigations are based on the pollen morphology of 50 species representing 20 genera of the family Rubiaceae by light and scanning electron microscope.

Materials and Methods

Polleniferous material was obtained from Karachi University Herbarium (KUH) or fresh material was collected from the field. The list of voucher specimens is deposited in KUH. The pollen grains were prepared for light (LM) and scanning microscopy (SEM) by the standard methods described by Erdtman (1952). For light microscopy, the pollen grains were mounted in unstained glycerin 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 SEM studies, pollen grains suspended in a drop of water were directly transferred with a fine pipette to a metallic stub using double sided cello tape and coated with gold in a sputtering chamber (Ion-sputter JFC-1100). Coating was restricted to 150°A. The S.E.M examination was carried out on a Jeol microscope JSM-2. The measurements are based on 15-20 readings from each specimen. Pollen length, polar axis (P) and equatorial diameter (E), aperture size and exine thickness were measured (Tables 1-5).

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

General pollen characters of the family Rubiaceae

Pollen grains usually radially symmetrical, isopolar. Mostly sub-prolate, to prolate-spheroidal rarely oblate-spheroidal or sub-oblate to prolate. Colpate to pantocolpate or colporate, sexine thicker or thinner than nexine. Tectal scabrate-punctate or reticulate to rugulate-reticulate, surface mostly spinulose or scabrate.

On the basis of exine ornamentation and apertural types, 9 distinct pollen types are recognized viz., *Argostemma sarmentosum*-type, *Aitchisonia rosea*-type *Galium elegans*-type, *Galium tenuissimum*-type, *Gaillonia macrantha*-type, *Jaubertia aucheri*-type, *Oldenlandia nudicaulis*-type, *Oldenlandia umbellata*-type and *Pseudogaillonia hymenostenphana*-type

Key to the pollen types

1. + Pollen grains colpate 2
 - Pollen grains colporate 7
2. + Tectum psilate *Argostemma sarmentosum* -type
 - Tectum not as above 3
3. + Tectum scabrate or spinulose *Galium tenuissimum*-type
 - Tectum not as above 4
4. + Tectum scabrate-punctate *Galium elegans*-type
 - Tectum not as above 5
5. + Tectum foveolate *Gaillonia macrantha*-type
 - Tectum reticulate-rugulate or reticulate 6
6. + Tectum reticulate *Jaubertia aucheri*-type
 - Tectum reticulate-rugulate *Aitchisonia rosea*-type
7. + Tectum scabrate or spinulose *Oldenlandia nudicaulis*-type
 - Tectum not as above 8
8. + Tectum reticulate *Oldenlandia umbellata*-type
 - Tectum reticulate-rugulate *Pseudogaillonia hymenostenphana*-type

Pollen type: *Aitchisonia rosea*-type (Fig. 1A-F).

Pollen class: 3-colpate

P/E ratio: 100-102

Shape: Prolate-spheroidal rarely oblate-spheroidal

Apertures: Colpi long rounded acute ends.

Exine: Sexine thicker or thinner than nexine.

Ornamentation: Reticulate-rugulate or rugulate-reticulate.

Measurements: Size: Length = (25.5-) 39.8 ± 0.2 (-53.8) μm and breadth (24.75) 39.5 ± 0.11 (54.74) μm , colpi (14.0-) 23.5 ± 0.42 (32.8) μm long. Mesocolpium 17.5-32.5 μm . Apocolpium c. 8.25-17.5 μm . Exine 2.25 (4.0 ± 0.5) 5.75 μm thick, sexine thicker than nexine. Tectum reticulate-rugulate or rugulate-reticulate.

Species included: *Aitchisonia rosea* Hemsl ex Aitch., *Leptodermis virgata* Edgew. and *Spermadictyon suaveolens* Roxb.

Key to the species

1. + Pollen grains oblate-spheroidal *Aitchisonia rosea*
 - Pollen grains prolate-spheroidal 2
2. + Colpi 21-28 μm in length *Leptodermis virgata*
 - Colpi 26-32 μm in length *Spermadictyon suaveolens*

Pollen type: *Argostemma sarmentosum*-type

Pollen class: 3-colpate

P/E ratio: 111

Shape: Prolate-spheroidal.

Apertures: Colpus long narrow with acute ends.

Exine: Sexine thinner than nexine.

Ornamentation: Psilate

Measurements: Size: Length = (7.49) 7.24 ± 1.98 (-8.25) μm and breadth (7.9 5) 7.18 ± 0.13 (7.18) μm , colpi (3.59-) 3.66 ± 0.51 (4.2) μm long. Mesocolpium μm (3.59-) 6.77 ± 0.26 (7.18). Apocolpium (3.23-) 3.54 ± 0.61 (3.59) μm . Exine 1.12- (1.71 ± 0.69) 3.59 μm thick, sexine thinner than nexine. Tectum psilate.

Species included: *Argostemma sarmentosum* Wall.

Pollen type: *Galium elegans*-type (Fig. 2 A-F, Fig. 3 A-D).

Pollen class: 5-10-colpate

P/E ratio: 120-123

Shape: Sub-prolate to prolate-spheroidal

Apertures: Colpus long narrow with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation: Scabrate-punctate

Measurements: Size: Length = (14.5-) 20.8 ± 0.2 (-27.8) μm and breadth (10.75) 14.5 ± 0.11 (19.74) μm , colpi (10.0-) 17.5 ± 0.42 (25.8) μm long. Mesocolpium 12.5-17.5 μm . Apocolpium c. 1.25-8.5 μm . Exine 1.25 (1.97 ± 0.5) 2.75 μm thick, sexine thicker than nexine. Tectum scabrate-punctate.

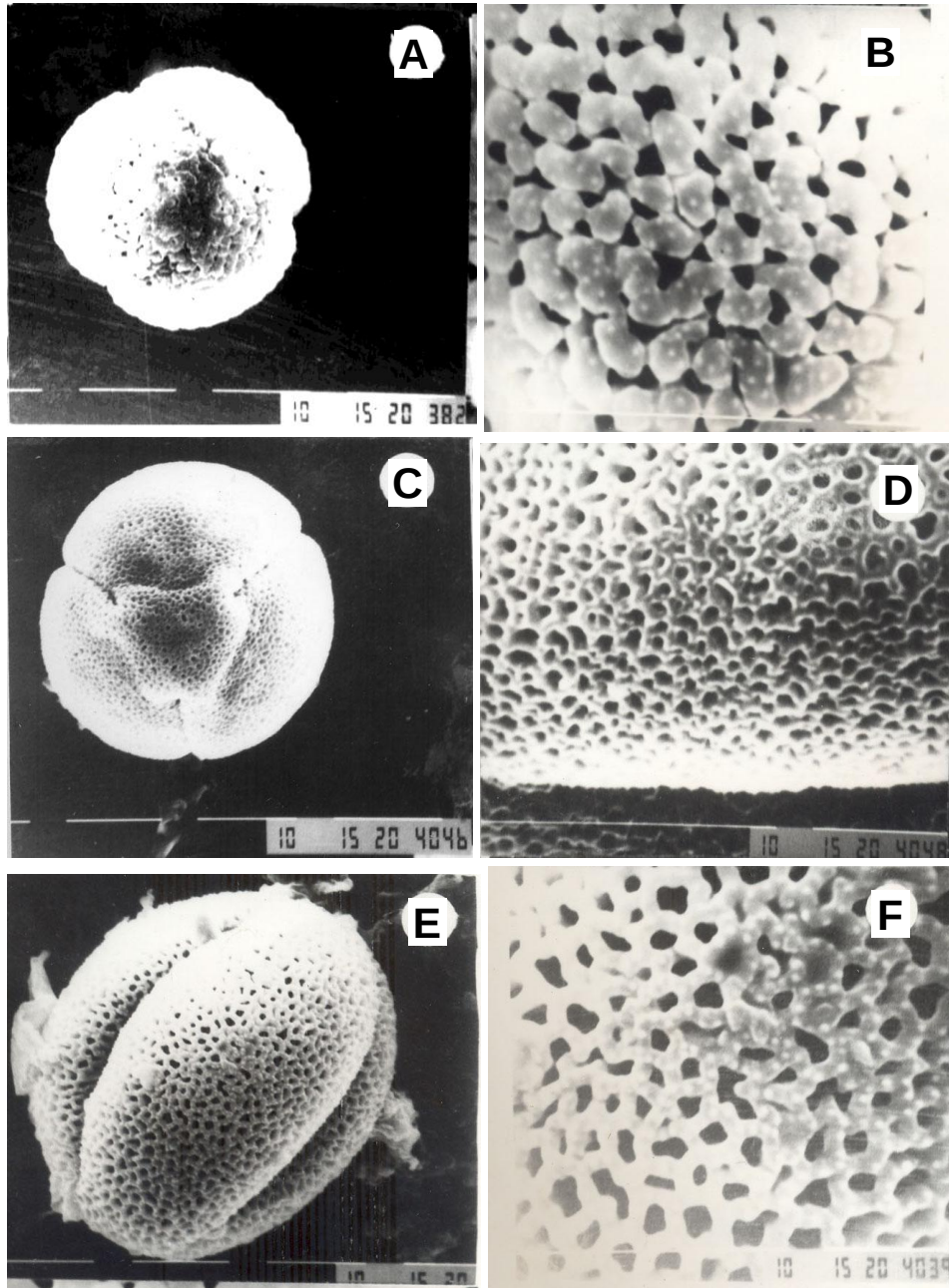


Fig. 1. Scanning Electron micrographs of pollen grains. *Aitchisonia rosea*: A, Polar view, B, Exine pattern. *Leprodermis virgata*: C, Polar view, D, Exine pattern. *Spermadictyon suaveolensa*: E, Equatorial view, F, Exine pattern.

Scale bar = A, C, E= 10 μ m. B, D & F= 1 μ m

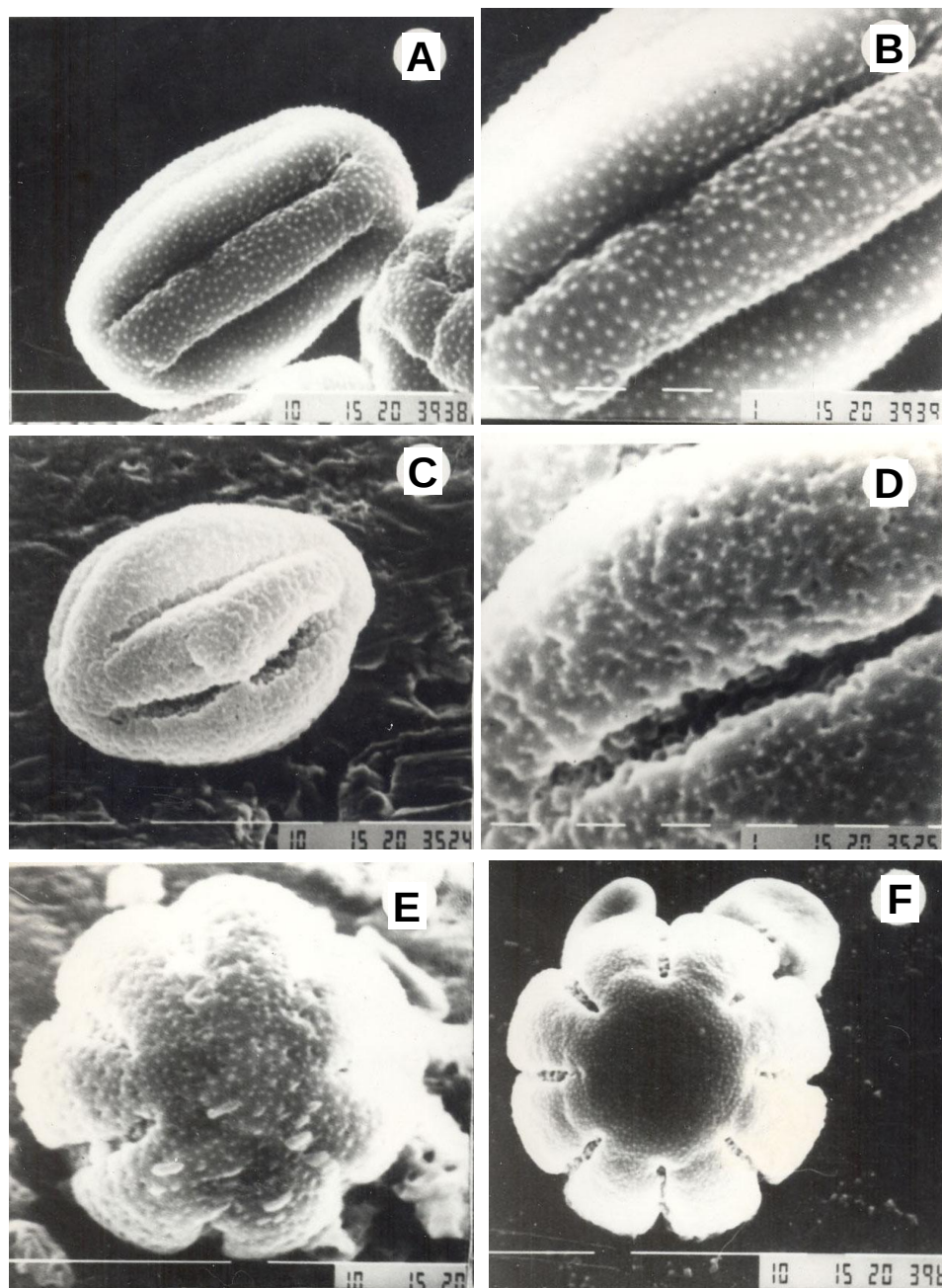


Fig. 2. Scanning Electron micrographs of pollen grains. *Galium asperuloides*: A, Equatorial view; B, Exine pattern. *Rubia chitralensis*: C, Equatorial view, D, Exine pattern. *Rubia cordifolia*: E, Polar view, *Galium tricornutum* F, Polar view. Scale bar = A, C, E & F = 10 μ m. B & D = 1 μ m

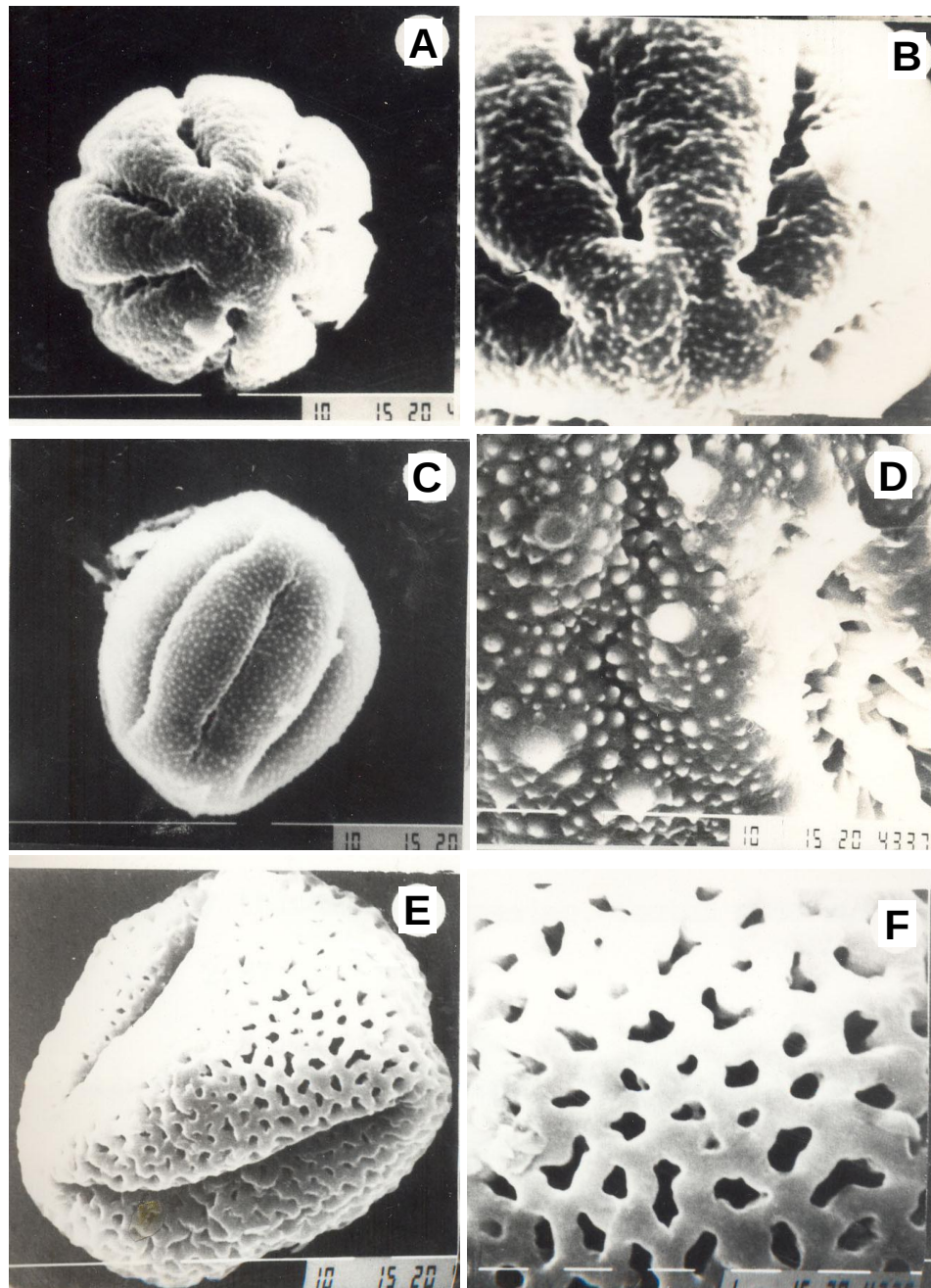


Fig.3. Scanning Electron micrographs of pollen grains. *Asperula oppositifolia*: A, Polar view; B, Exine pattern. *Asperula karataviensis*: C, Equatorial view, D, Exine pattern. *Pterogaillonia calycoptera*: E, Equatorial view, F, Exine pattern. Scale bar = A, C, E= 10 μ m. B, D & F= 1 μ m

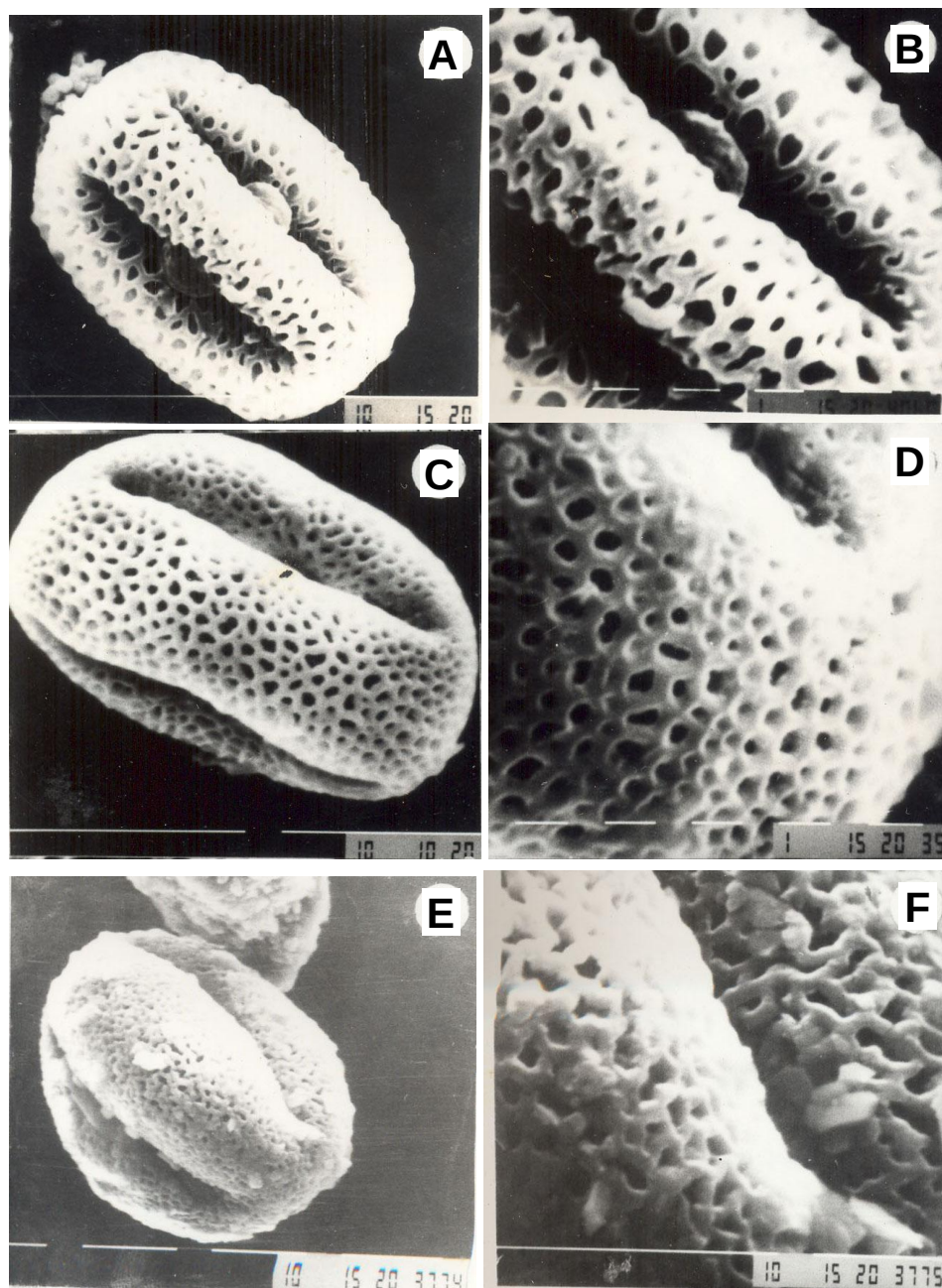


Fig. 4. Scanning Electron micrographs of pollen grains. *Kohautia gracilis*: A, Equatorial view; B, Exine pattern. *Oldenlandia umbellata*: C, Equatorial view, D, Exine pattern. *Pseudogaillonia hymenostephana*: E, Equatorial view, F, Exine pattern. Scale bar = A, C, E= 10 μ m. B, D & F= 1 μ m

Species included: *Asperula karataviensi* Pavl., *A. oppositifolia* Reg. & Schmalh., *Rubia cordifolia* L., *R. infundibularis* Hemsl. & Lace, *R. kurramensis* Nazim, *R. tinctorum* L., *R. chitralensis* Ehrend., *R. tibetica* Hook.f., *Callipeltis cucullaris* (L.) Rothm., *Galium tricornutum* Dandy, *G. saturejifolium* Trev., *G. verum* L., *G. boreale* L., *G. elegans* Wall., *G. decasnei* Boiss., *G. asperuloides* Edgew., *G. aparine* L., *G. subfalcatum* Nazim. & Ehrend., *Gaillonia asperuliformis* Lincz., *Asperula setose* Jaub. & Spech, *Asperula oppositifolia* Reg. & Schmalh., *Galium arperifolium* Wall., *G. chitralensis* Nazim., *Galium serpylloides* Royle ex Hook.f., *Galium setaceum* Lamk., *Galium acutum* Edgew., *Galium tetraphyllum* Nazim. & Ehrend.

Key to the species group

1. + Pollen grains Sub-prolate Group-1
(*Asperula oppositifolia* Reg. & Schmalh., *R. infundibularis* Hemsl. & Lace, *R. kurramensis* Nazim, *R. tinctorum* L., *R. chitralensis* Eherd., *R. tibetica* Hook.f., *Galium verum* L., *G. boreale* L., *G. decasnei* Boiss., *G. subfalcatum* Nazim. & Ehrend., *Gaillonia asperuliformis* Lincz., *Asperula setose* Jaub. & Spech, *Asperula oppositifolia* Reg. & Schmalh., *Galium arperifolium* Wall., *Galium setaceum* Lamk., *Galium acutum* Edgew., *Galium tetraphyllum* Nazim. & Ehrend)
- Pollen grains prolate-spheroidal Group-2
(*Asperula karataviensi* Pavl., *Rubia cordifolia* L., *Callipeltis cucullaris* (L.) Rothm., *Galium tricornutum* Dandy, *G. saturejifolium* Trev., *G. elegans* Wall., *G. asperuloides* Edgew., *G. aparine* L., *Gaillonia asperuliformis* Lincz., *Asperula setose* Jaub. & Spech, *G. chitralensis* Nazim., *Galium serpylloides* Royle ex Hook.f.)

Pollen type: *Galium tenuissimum*-type

Pollen class: 5-10-colpate

P/E ratio: 1.26

Shape: Prolate-spheroidal.

Apertures: Colpus long narrow with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation: Spinulose-punctate

Measurements: Size: Length = (15.5) 20.8 ± 0.2 (-25.5) μm and breadth (14.75) 11.86 ± 0.11 (22.5) μm , colpi (7.5-) 16.25 ± 0.42 (17.5) μm long. Mesocolpium 7.5-8.75 μm . Apocolpium 0.5 μm . Exine 1.25- (1.62 ± 0.5) 2.0 μm thick, sexine thicker than nexine.

Tectum spinulose-punctate

Species included: *Galium hirtiflorum* Requeen ex DC., *G. tenuissimum* M. Bieb.

Key to the species

1. + Pollen grains 8-10-colpate *Galium tenuissimum*
- Pollen grains 5-colpate *Galium hirtiflorum*

Pollen type: *Gaillonia macrantha*-type

Pollen class: 3-colpate

P/E ratio: 111

Shape: Prolate-spheroidal

Apertures: Colpus long narrow with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation: Foveolate

Measurements: Size: Length = (39.49) 44.33 ± 1.98 (-50.67) μm and breadth (35.9 5) 39.75 ± 0.11 (46.65) μm , colpi (28.72–) 31.75 ± 1.63 (39.48) μm long. Mesocolpium μm (25.13–) 29.43 ± 0.05 (32.31). Apocolpium, (1.79–) 4.48 ± 0.61 (7.18) μm . Exine 3.23–(3.4 $\pm$ 0.5) 3.59 μm thick, sexine thicker than nexine. Tectum foveolate.

Species included: *Gaillonia macrantha* Blatt. & Hallb. (Fig. 3E & F).

Pollen type: *Jaubertia aucheri*-type (Fig. 3E & F).

Pollen class: 3-5-colpate

P/E ratio: 1.00-1.06

Shape: Prolate-spheroidal or oblate-spheroidal

Apertures: Colpus long narrow with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation: Reticulate

Measurements: Size: Length = (32.5) 21.8 ± 0.2 (-41.5) μm and breadth (30.75) 28.75 ± 0.11 (39.5) μm , colpi (17–) 30.75 ± 0.42 (25.8) μm long. Mesocolpium 10.5-25 μm . Apocolpium 7.1-14 μm . Exine 3.23–(3.4 $\pm$ 0.5) 3.5 μm thick, sexine thinner than nexine. Tectum reticulate.

Species included: *Jaubertia aucheri* Guill., *Leptodermis lanceolata* Wall., *Pterogaillonia calycoptera* (Decne.) Lincz, *Kohautia aspera* (Roth.) Bremek, *K. retrosa* (Boiss.) Bremek, *Gaillonia afghanica* Ehrend.

Key to the species

1. + Pollen grains prolate *Gaillonia afghanica*
 - Pollen grains prolate-spheroidal, rarely oblate-spheroidal 2
2. + Pollen grains oblate-spheroidal *Leptodermis lanceolata*
 - Pollen grains prolate-spheroidal, rarely oblate-spheroidal Group-I
 (*Jaubertia aucheri* Guill., *Pterogaillonia calycoptera* (Decne.) Lincz, *Kohautia aspera* (Roth.) Bremek, *K. retrosa* (Boiss.) Bremek)

Pollen type: *Oldenlandia nudicaulis*-type (Fig. 2 A& B).

Pollen class: 3-4-colporate.

P/E ratio: 111

Shape: Prolate-spheroidal.

Apertures: Colpus long narrow with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation: Spinulose-punctate

Measurements: Size: Length = (30.5) 31.78 ± 0.53 (-33.75) μm and breadth (25.85) 28.86 ± 0.11 (31.5) $31.25 \mu\text{m}$, colpi (17.75–) 19.92 ± 0.42 (21.8) μm long. Mesocolpium (17.75–) 19.37 ± 1.87 (25.8) μm . Apocolpium 5-7.5 μm . Exine 2.25–(2.46 $\pm$ 0.06) 2.75 μm thick, sexine thinner than nexine. Tectum Spinulose-punctate.

Species included: *Oldenlandia nudicaulis* Roth

Pollen type: *Oldenlandia umbellata*-type (Fig. 4 A-D).

Pollen class: 3-5-colporate,

P/E ratio: 88-1.35

Shape: Sub-prolate and prolate-spheroidal

Apertures: Colpus long narrow with acute ends.

Exine: Sexine thicker or thinner than nexine.

Ornamentation: Reticulate

Measurements: Size: Length = (14-) 35.75 ± 0.2 (-57.5) μm and breadth (13.75) 18.6 ± 0.11 (64.5) μm , colpi (10.8-) 29.75 ± 0.42 (18.5) μm long. Mesocolpium 7.12 (12.05 $\pm$ 0.25) 17.6 μm . Apocolpium 1.9 (7.25 $\pm$ 1.24) 12.02 μm . Exine 1.75 (4.77 $\pm$ 0.5) 7.8 μm thick, sexine thicker than nexine. Tectum reticulate.

Species included: *Oldenlandia umbellata* L., *O. corymbosa* L., *Kohautia gracilis* (Wall.) DC., *Borreria articularis* (L.f.) F.N. Williams and *Wendlandia exerta* (Roxb.) DC. *Himalrandia tetrasperma* (Roxb.) Yamazaki, *Catunaregaum spinosa* (Thunb.) Tirvengadam, *Pavetta tomentosa* Roxb. ex Smith, *Mitragyna parvifolia* (Roxb.) Korth.

Key to the species

1. + Pollen grains subprolate to prolate-spheroidal 3
 - Pollen grains sub-oblate to oblate-spheroidal 2
2. + Pollen grains sub-oblate *Borreria articularis*
 - Pollen grains oblate-spheroidal Group-I
 (*Pavetta tomentosa*, *Mitragyna parvifolia*)
3. + Pollen grains prolate-spheroidal *O. corymbosa*
 - Pollen grains subprolate Group-II
 (*Oldenlandia umbellata* L., *Kohautia gracilis* (Wall.) DC and *Wendlandia exerta* (Roxb.) DC. *Himalrandia tetrasperma* (Roxb.) Yamazaki, *Catunaregaum spinosa* (Thunb.) Tirvengadam)

Pollen type: *Pseudogallonia hymenostephana*-type (Fig. 4E & F).

Pollen class: 6-7-colporate.

P/E ratio: 1.26

Shape: Sub-prolate.

Apertures: Colpus long narrow with acute ends.

Exine: Sexine slightly thicker than nexine.

Ornamentation: Rugulate-reticulate

Measurements: Size: Length = (33.38) 38.61 ± 0.2 (-43.5) μm and breadth (25.13) 30.75 ± 0.19 (35.9) μm , colpi (25.84-) 25.84 ± 0.52 (28.8) μm long. Mesocolpium 25.31 (25.64 $\pm$ 1.79) 28.72 μm . Apocolpium 7.18 (9.87 $\pm$ 2.7) 12.56 μm . Exine 2.87- (3.33 $\pm$ 0.10) 3.59 μm thick, sexine slightly thinner than nexine. Tectum rugulate-reticulate, lumina 0.14-1.25 μm in diameter.

Species included: *Pseudogallonia hymenostephana* (Jaub. & Spach) Lincz.

Discussion

Rubiaceae is known for its difficult intrafamilial classification. Schumann (1891) divided the Rubiaceae into two subfamilies, Cinchonideae and Coffeoideae based on a single characters, the numbers of ovules per locules. Bremekamp (1954, 1966) recognized eight subfamilies. Robbrecht (1982), divided the family Rubiaceae into 4 subfamilies Cinchonoideae, Ixoroideae, Rubioideae and Antirheoideae. The position of Rubiaceae in the order Gentiales was first suggested by Utzschneider (1947) and later established by Wagenitz (1959, 1964). This position is accepted by most taxonomists dealing with higher-level classification (Dahlgren, 1980. Thorne, 1983, 1992, Takhtajan (1987) and is also supported by molecular and morphological data (Downie & Palmer, 1992) Chase *et al.*, 1993 and Olmstead *et al.*, 1993.

According to molecular basis, the Rubiaceae are monophyletic and are a sister group to the rest of the Gentianales, rbcL sequencing is very useful for Phylogenetic analysis.

Rubiaceae seems to be very suitable for palynological studies. It has remarkable ecological, economical and taxonomical importance. It is heterogeneous enough from palynological point of view and exine structure also shows a remarkable morphological variation.

In the present palynological studies of Rubiaceae more frequent aperture type is colpate, although colpate type has also been found. In colpate grains aperture number varies from 3-11 colpate, among which 6-7 colpate are more commonly found. Among colpate grains 3-colpate grains are mostly present but *Borreria* is the exceptional case in which colporae varies from 11-13.

Oras are of two type i.e., la-longate and 10-longate. There are 4 different grains shape found, which are sub-oblate, oblate-spheroidal, sub-prolate and prolate-spheroidal. More frequent types are sub-prolate and prolate-spheroidal. Sub-oblate shape is found only in *Borreria articularis*. Grains are more commonly fossaperturate in polar view. P.A.I. varies from 1.058-4.262. Most frequent tectum type found in these species is scabrate-punctate but spinulose, rugulate and reticulate tectum is also found. Foveolate and psilate types of tectum are found in *Gaillonia macrantha* and *Argostemma surmentosum* respectively.

Pollen morphology of Rubiaceae does not support the infrafamilial classification. The genera belonging to the tribe Rubieae such as the species of *Galium* L., and *Rubia* L., fall in different pollen types. Similarly two genera i.e., *Khautia* Cham. & Schlecht. and *Oldenlandia* L., belonging to the tribe Hedyotideae are distributed in three different pollen types. Even three species of *Oldenlandia* L., viz. *Oldenlandia corymbosa* L., *O. nudicaulis* Roth, *O. umbellata* L. fall under two different pollen types. However, present palynological data supports the placement of the family Rubiaceae with Apocynaceae, Loganiaceae and Gentianaceae in the order Gentianales by Dahlgren (1989). Rubiaceae pollen are more or less similar to the pollen of Apocynaceae, Gentianaceae, and Loganiaceae. In all these families colpate pollen are common with reticulate or scabrate tectum (Utzschneider, 1951, Erdtman, 1952 and Qaiser & Perveen, 1997).

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