

POLLEN FLORA OF PAKISTAN – L. RANUNCULACEAE

ANJUM PERVEEN AND MUHAMMAD QAISER

*Department of Botany,
University of Karachi, Karachi-75270, Pakistan.*

Abstract

Pollen morphology of 27 species representing 10 genera of the family Ranunculaceae from Pakistan has been examined by light and scanning electron microscope. Pollen grains usually radially symmetrical, isopolar rarely apolar. Mostly sub-prolate, often oblate-spheroidal to prolate, colpate or pantocolpate, rarely pantoporate, sexine thicker or thinner than nexine. Tectal surface mostly spinulose or scabrate often striate or verucate. On the basis of exine ornamentation and apertural types, 5 distinct pollen types are recognized viz., *Clematis grata*-type, *Delphinium denudatum*-type, *Ranunculus muricatus*-type *Thalictrum isopyroides*-type and *Trollius acaulis*-type.

Introduction

Ranunculaceae is a large family of c. 50 genera and c. 2000 species widely distributed throughout the northern hemisphere but also in southern temperate regions (Mabberley 1987). In Pakistan, it is represented by 22 genera and c. 114 species, of which genera like *Nigella*, *Anemone*, *Aquilegia* include plants of ornamental value (Riedl & Nasir 1991). Cronquist (1968), Thorne (1968) and Takhtajan (1969) treated this family under the order Ranunculales.

Plant mostly herbs, some times lianoid shrub or vines (*Clematis*), flower bisexual actinomorphic or zygomorphic, corolla 5-many petals, rarely absent (*Thalictrum*). Mostly the genera are insect pollinated, except *Thalictrum* which is wind pollinated.

Pollen morphology of some genera of the family Ranunculaceae has been examined by Erdtman (1952), Vishnu-Mittre Sharma (1962), Boet & Spoil (1968), Huyna (1970), Nowicke (1975), Kuprianova & Alyoshina (1978), Mkrtchyan & Agalabyan (1978), Skvarla & Nowicke (1979), Nowicke & Skvarla (1977, 1979, 1983). There are no reports on pollen morphology of the family Ranunculaceae from Pakistan. Present investigations are based on the pollen morphology of 27 species representing 10 genera of the family Ranunculaceae by light and scanning electron microscope.

Materials and Methods

Pollen samples were obtained from Karachi University Herbarium (KUH) or 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 diameter, polar axis (P) and equatorial diameter (E), aperture size and exine thickness were measured (Table 1-3).

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

General pollen characters of the family Ranunculaceae

Pollen grains usually radially symmetrical, isopolar rarely apolar. Mostly sub-prolate, often oblate-spheroidal to prolate, colpate to pantocolpate, rarely pantoporate, sexine thicker or thinner than nexine. Tectal surface mostly spinulose or scabrate often striate or verrucate.

On the basis of exine ornamentation and apertural types, 5 distinct pollen types viz., *Clematis grata*-type, *Delphinium denudatum*-type, *Ranunculus muricatus*, *Thalictrum isopyroides*-type and *Trollius acaulis*-type are recognized.

Key to the pollen types

- 1 + Pollen grains pantoporate *Thalictrum isopyroides*-type
- Pollen grains tricolpate rarely tetracolpate 2
- 2 + Tectum striate *Trollius acaulis*-type
- Tectum not as above 3
- 3 + Tectum verrucate *Ranunculus muricatus*
- Tectum scabrate or spinulose 4
- 4 + Tectum scabrate *Delphinium denudatum* -type
- Tectum spinulose *Clematis grata*- type

Pollen type: *Clematis grata*-type (Fig. 1A-F; Fig. 2A-E).

Pollen class: 3-Colpate.

P/E ratio: 0.88-1.35.

Shape: Prolate-spheroidal, sub-prolate to prolate.

Apertures: Ectocolpus long narrow with acute ends.

Exine: Sexine thicker or thinner than nexine.

Ornamentation: Spinulose.

Measurements: Size: Length = (15-) 20.8 ± 0.2 (-26.5) μm and breadth (17.75) 18.6 ± 0.11 (19.5) μm , colpi (15.8-) 16.75 ± 0.42 (17.5) μm in long. Mesocolpium 5.6 (12.13 \pm 0.25) $18.6 \mu\text{m}$. Apocolpium 1.25 (2.0 \pm 1.24) 2.02 μm . Exine 1.75 (2.18 \pm 0.5) 2.5 μm thick, sexine thicker than nexine. Tectum spinulose.

Species included: *Aconitum chasmanthum* Stapf ex Holmes, *Adonis aestivalis* L., *Aquilegia pubiflora* Wall. ex Royle, *Anemone vitifolia* Buch.-Ham. *Caltha alba* Camb., *Delphinium vestitum* Wall. ex Royle, *Clematis gouriana* Roxb. ex DC., *C. grata* Wall., *C. connata* DC., *C. aspleniifolia* Schrenk., *C. graveolens* Lindl., *C. orientalis* L., *Ranunculus sceleratus* L., *R. hirtellus* Royle, *R. munroanus* Drum. ex Dunn. *R. brotherusii* Freyn.

Table 1. General pollen characters of species included in pollen type *Clematis grata*.

Name of species	Shape	Polar axis μm (P)	Equatorial diameter μm (E)	Colpus length μm	Exine thickness μm	Tectum
<i>Aquilegia pubiflora</i> Wall ex Royle	Prolate	20.0 (21.59 \pm 0.32) 24.5	14.0 (15.91 \pm 0.29) 17.75	12.5 (16.04 \pm 0.35) 18.75	2.0 (2.29 \pm 0.04)	2.5
<i>Aconitum chashmanthum</i> Stierp ex Holmes	Pr-Sp	22.5 (24.56 \pm 0.62) 27.5	19.5 (22.68 \pm 0.74) 25.0	17.5 (19.53 \pm 0.40) 21.25	1.75 (2.15 \pm 0.09) 2.5	Sp
<i>Adonis aestivalis</i> L.	Sub-Pr	23.47 (24.47 \pm 0.22) 25.0	17.5 (18.93 \pm 0.34) 20.5	15.0 (17.5 \pm 0.53) 20.0	2.0 (2.25 \pm 0.067) 2.5	Sp
<i>Anemone vitifolia</i> Buch.-Ham.	Pr-Sp	18.75 (21.82 \pm 0.57) 25.0	18.75 (20.85 \pm 0.65) 25.0	12.5 (13.75 \pm 0.41) 15.0	2.0 (2.44 \pm 0.05)	2.5
<i>Callitha alba</i> Camb.	Sp	30.0 (32.5 \pm 0.67) 35.0	-	7.5 (8.39 \pm 0.45) 10.0	1.75 (2.10 \pm 0.92) 2.5	Sp
<i>Clematis asplenifolia</i> Schrenk	Sub-Pr	17.5 (19.65 \pm 0.24) 22.5	15.0 (16.99 \pm 0.20) 49.6	12.5 (14.86 \pm 0.17) 17.5	1.75 (2.16 \pm 0.03) 2.5	Sp
<i>Clematis connata</i> DC	Sub-Pr	22.5 (24.89 \pm 0.20) 27.5	17.5 (19.2 \pm 0.317) 22.5	17.5 (18.5 \pm 0.21) 10.0	1.75 (2.44 \pm 0.9)	2.5
<i>Clematis gouriana</i> Roxb. ex DC	Ob-Sp	15.75 (17.89 \pm 0.45) 19.25	17.5	10.0 (11.0 \pm 0.37) 12.5	1.75 (2.07 \pm 0.76) 2.25	Sp
<i>Clematis grata</i> Wall.	Ob-Sp	26.75 (18.0 \pm 0.23) 20.0	18.75 (20.4 \pm 0.29) 22.5	10.2 (11.2 \pm 0.30) 12.5	1.75 (2.17 \pm 0.048) 2.5	Sp
<i>Clematis graveolens</i> Lindl.	Pr-Sp	21.25 (22.75 \pm 0.22) 25.0	18.75 (21.36 \pm 0.36) 23.75	15.0 (16.81 \pm 0.24) 17.5	2.0 (2.38 \pm 0.039) 2.5	Sp
<i>Clematis orientalis</i> L.	Pr	21.25 (24.47 \pm 0.44) 26.25	16.75 (18.38 \pm 0.37) 20.0	16.25 (19.54 \pm 0.59) 22.5	1.75 (2.09 \pm 0.06) 2.25	Sp
<i>Delphinium vestitum</i> Wall. ex Royle	Sub-Pr	25.7 (27.20 \pm 0.48) 28.75	20.0 (22.54 \pm 0.66) 23.75	-	1.25 (1.7 \pm 0.16) 2.25	Sp
<i>Ranunculus brotherusii</i> Freyn	Sub-Pr	23.0 (24.95 \pm 0.86) 28.5	17.5 (19.7 \pm 0.68) 22.5	17.5 (20.0 \pm 0.047) 22.5	2.0 (2.25 \pm 0.09)	2.5
<i>Ranunculus hirtellus</i> Royle	Sub-Pr	22.0 (24.67 \pm 0.34) 26.75	17.5 (20.15 \pm 0.67) 25.0	17.5 (19.37 \pm 0.33) 20.0	1.75 (2.3 \pm 0.08)	2.5
<i>Ranunculus munroanus</i> Drun. ex Dunn	Sub-Pr	22.5 (25.0 \pm 0.42) 25.5	17.5 (19.36 \pm 0.38) 20.5	17.5 (19.86 \pm 0.50) 22.5	2.25 (2.37 \pm 0.04) 2.5	Sp
<i>Ranunculus sceleratus</i> L.	Sub-Pr	25.0 (27.43 \pm 0.36) 30.0	20.0 (22.85 \pm 0.39) 25.0	2.0 (2.285 \pm 0.39) 25.0	2.0 (2.08 \pm 0.03) 2.25	Sp

Ob-Pr= Oblate-spheroidal, Pr= Prolate, Sub-Pr= Sub-prolate, Pr-Sp= Prolate-Speroidal, Sp=Spinulose

Table 2. General pollen characters of species included in pollen type *Delphinium denudatum*.

Name of species	Shape	Polar axis μm (P)	Equatorial diameter μm (E)	Colpus length μm	Exine thickness μm	Tectum
<i>Aconitum larve</i> Royle	Sub-Pr	27.5 (28.58 \pm 0.45) 30.0	22.0 (22.5 \pm 0.60)	17.5 (20.0 \pm 0.60) 20.0	1.75 (2.04 \pm 0.076)	Scabrate
<i>Delphinium aquilegifolium</i> (Boiss.) Bomm.	Sub-Pr	21.25 (23.02 \pm 0.32) 25.0	17.25 (18.67 \pm 0.35) 21.25	-	1.25 (1.45 \pm 0.04) 1.75	Seb.
<i>D. denudatum</i> Wall. ex Hook.f. & Thoms.	Sub-Pr	26.0 (26.75 \pm 0.50) 27.25	21.75 (22.1 \pm 0.37) 22.5	c. 21.25 (21.67 \pm 6.6) 22.5	1.75	Seb
<i>Ranunculus laetus</i> Wall.	Pr-Sp	20.5 (28.05 \pm 0.43)	23.25 (24.5 \pm 0.53) 29.5	10.0 (11.6 \pm 0.40) 15.0	2.25 (2.40 \pm 0.017) 2.5	Seb

Ob-P= Oblate-spheroidal, Sub-Pr= Subprolate, Pr-Sp= Prolate-Spheroidal

Table 3. General pollen characters of species included in pollen type *Thalictrum isopyroides*.

Name of species	No. of pore	Polar axis μm (P)	Equatorial diameter μm (E)	Colpus length μm	Exine thickness μm	Tectum
<i>Thalictrum pedunculatum</i> Edgew.	10	18.75 (22.04 \pm 0.75) 23.75	-	3.75 (4.45 \pm 0.198) 5.0	1.75 (1.95 \pm 0.04) 2.0	Sp
<i>Thalictrum cultratum</i> Wall.	6-10	18.25 (19.85 \pm 0.23) 20.0	-	3.0 (4.06 \pm 0.19) 5.25	1.75 (2.0 \pm 0.04) 2.25	Sp
<i>Thalictrum isopyroides</i> C.A. Mey.	Panto	17.50 (18.95 \pm 0.25) 20.0	-	17.5 (18.78 \pm 0.31) 20.0	1.5 (1.9 \pm 0.10) 2.5	Sp-Scab
<i>Thalictrum minus</i> L.	8-12	17.5 (19.67 \pm 0.34) 21.5	-	3.0 (4.25 \pm 0.207) 5.0	1.75 (2.02 \pm 0.05) 2.25	Sp
<i>Ranunculus afghanicus</i> Aitch- & Hemsl.		26.25 (30.29 \pm 0.6) 33.75	25.0 (32.5 \pm 0.40)	32.0 7.0	3.75 (5.08 \pm 0.182) 2.3 (2.47 \pm 0.03)	3.0 Sp

Ob-Sp= Oblate-spheroidal, Pr=Prolate, Sub-Pr= Subprolate, Scab= Scabrate, Panto= Pantoporate

Fig. 1. Scanning Electron Micrographs of pollen grains. *Aquilegia pabiflora*: A, Equatorial view, B, Exine pattern. *Caltha alba*: C, Pollen grain, D, Exine pattern. *Anemone vitifolia*: E, Polar view, F, Exine pattern
Scale bar =A, B, D-F = 10 μ m. C= 1 μ m

Fig. 2. Scanning Electron micrographs of pollen grains. *Clematis gouriana*: A, Equatorial view; B, Polar view, C, Exine pattern. *Ranunculus munroanus*: D, Equatorial view, E, Exine pattern. *Aconitum laeve*: F, Equatorial view.
Scale bar = A-E = 10 μ m. F= 1 μ m

Key to the species

- 1 + Pollen grains subprolate to prolate 3
 - Pollen grains prolate-spheroidal to oblate-spheroidal 2
 2 + Pollen grains prolate-spheroidal group-1
 (*Anemone vitifolia*, *Clematis graveolens*),
 - Pollen grains oblate-spheroidal group-2
 (*Clematis gouraina*, *C. grata*)
 3 + Pollen grains prolate group-3
 (*Aquilegia pubiflora*, *Clematis orientalis*)
 - Pollen grains subprolate group-4

Adonis aestivalis L. Buch.-Ham. *Caltha alba* Camb., *Delphinium vestitum* Wall. ex Royle, *C. connata* DC., *C. aspleniifolia* Schrenk, *Ranunculus sceleratus* L., *R. brotherusii* Freyn, *R. hirtellus* Royle, *R. munroanus* Drum. ex Dunn.

Pollen type: *Delphinium denudatum*-type (Fig. 2F; Fig. 3-A-F).

Pollen class: Tricolpate.

P/E ratio: 1.20-1.23.

Shape: Sub-prolate to prolate or prolate-spheroidal.

Apertures: Ectocolpus long narrow with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation: Scabrate.

Measurements: Size: Length = (21.5-) 26.8 ± 0.2 (-31.5) μm and breadth (17.75) 23.5 ± 0.11 (29.5) μm , colpi (10.0-) 17.5 ± 0.42 (25.8) μm in long. Mesocolpium 12.5-17.5 μm . Apocolpium c. 1.25-8.5 μm . Exine 1.25 (2.0 ± 0.5) 2.75 μm thick, sexine thicker than nexine. Tectum scabrate.

Species included: *Aconitum laeve* Royle., *Delphinium aquilegifolium* (Boiss.) Bornm., *D. denudatum* Wall. ex Hook. f. & Thoms., *Ranunculus laetus* Wall.

Pollen type: *Thalictrum isopyroides*-type - (Fig. 4A&B).

Pollen class: 6-12 or pantoporate.

P/E ratio: 100-102.

Shape: Spheroidal rarely oblate-spheroidal.

Apertures: Pore small rounded.

Exine: Sexine thicker or thinner than nexine.

Ornamentation: Spinulose.

Measurements: Size: Diameter of pollen = $17.5 (26 \pm 1.2) 35$, and pore 1.75-3.25 μm in diameter. Exine 1.5-2.25 μm thick, sexine thicker or thinner nexine. Tectum spinulose.

Species included: *Thalictrum cultratum* Wall., *T. isopyroides* C.A. Mey, *T. pedunculatum* Edgew., *T. minus* L., *Ranunculus afghanicus* Aitch. & Hemsl.

Key to the species

- 1 + Pollen grains pantoporate *Thalictrum isopyroides*
 - Pollen grains 6-12 porate group-1
 Thalictrum cultratum, *T. minus*
 T. pedunculata, *Ranunculus afghanicus*

Fig. 3. Scanning Electron Micrographs of pollen grains: *Aconitum laeve*: A, Exine pattern. *Delphinium denudatum*: B, Polar view, C, Exine pattern, D, Equatorial view. *Ranunculus laetus*: E, Polar view, F, Exine pattern
Scale bar = B, D, & E = 10; A, C, F = 1 μm

Fig. 4. Scanning Electron Micrographs of pollen grains: *Thalictrum isopyroides*: A, Pollen grain, B, Exine pattern. *Trollius acualis*: C, Polar view, D, Exine pattern
Scale bar = A & C = 10; B & D = 1 μ m

Pollen type: *Ranunculus muricatus* -type

Pollen class: 3-colpate.

P/E ratio: 1.06.

Shape: Prolate-spheroidal.

Apertures: Ectocolpus long narrow with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation: Scabrate-verucate.

Measurements: Size: Length = (33.5) 37.8 ± 0.2 (-42.5) μ m and breadth (35) 28.75 ± 0.11 (40.5) μ m, colpi (15-) 30.75 ± 0.42 (17.5) μ m in long. Mesocolpium 22.5 μ m. Apocolpium 1.5- 2.5 μ m. Exine 2-5- (2.16 ± 0.5) 3.05 μ m thick, sexine thinner than nexine. Tectum verrucate-scabrate.

Species included: *Ranunculus muricatus* L.

Pollen type: *Trollius acualis*-type (Fig. 4C&D).

Pollen class: 3-colpate.

P/E ratio: 1.26.

Shape: Subprolate.

Apertures: Ectocolpus long narrow with acute ends.

Exine: Sexine thicker than nexine.

Ornamentation: Striate.

Measurements: Size: Length = (36.5) 21.8 ± 0.2 (-37.5) μm and breadth (2475) 28.75 ± 0.11 (32.5) μm , colpi (27-) 30.75 ± 0.42 (32.8) μm in long. Mesocolpium 22.5 μm . Apocolpium c. 2.5 μm . Exine 2-(2.08 \pm 0.5) 2.5 μm thick, sexine thinner than nexine. Tectum striate.

Species included: *Trollius acualis* Lindley.

Discussion

Ranunculaceae representing 27 species is distributed in 10 genera i.e., *Aquilegia*, *Caltha*, *Aconitum*, *Delphinium*, *Anemone*, *Clematis*, *Thalictrum*, *Adonis*, *Ranunculus*, *Trollius*. According to Nowicke & Skvarla (1979) the family Ranunculaceae is remarkably uniform with only exceptions to the spinulose and punctate, perforated tectum. Present studies also confirms the uniformity of pollen. However, considerable variation is observed in shape, apertural types, arrangement of apertures and tectal surface. Some species also have differences in the colpal surface, such as in *Aconitum laeve* Royle rugulate colpal surface with scabrate tectum is present while the other species of the same genus i.e., *A. chasmanthum* Stapf ex Holmes has spinulose tectum with granulated colpal surface. Present studies of Ranunculaceae revealed that the family has 4 pollen shape classes i.e., oblate-spheroidal, prolate-spheroidal, subprolate and prolate. Among these subprolate is the most frequent one and found in 12 species belonging to the genera *Aconitum*, *Delphinium*, *Clematis*, *Adonis* and *Ranunculus* whereas prolate subclass is most rare and found in *Aquilegia*. Three different apertural types are also found i.e., 3-colpate, pantocolpate and pantoporate, whereas tectum type is fairly uniform i.e., most of the species have spinulose tectum. However, in *Ranunculus muricatus* L., has scabrate-verucate tectum, while in *R. laetus* Wall., scabrate-verrucate tectum is present. *Trollius acaulis* Lindley is the only exception which has coarsely striated tectum with granulated colpal surface. In many taxa of Ranunculaceae pollen are same as of Centrospermous families like, Amaranthaceae, Caryophyllaceae, Chenopodiaceae and Nyctaginaceae (Nowicke & Skvarla 1979). Erdtman (1952) considered that Ranunculaceae has more or less similar pollen grains as in Alismataceae, Berberidaceae and Lardizabalaceae, which also supports the classification of Dahlgren (1989) and Cronquist (1968).

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