

## POLLEN FLORA OF PAKISTAN–LXXI. ONAGRACEAE

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## Abstract

Pollen morphology of three genera representing 13 species of the family Onagraceae from Pakistan has been examined by light and scanning electron microscope. Pollen grains usually tetrads rarely monads, radially symmetrical, isopolar, mostly oblate rarely spheroidal, porate sexine thicker or thinner than nexine. Tectal surface mostly foveolate-rugulate with viscin threads. On the basis of size and tectum types 3 distinct pollen types are recognized, viz., *Epilobium angustifolium*-type, *Circaea alpina*-type and *Oenothera affinis*-type.

## Introduction

Onagraceae is a well-defined family of about 17 genera and 675 species of temperate and subtropical distribution (Mabberley, 2008). The family is characterized by having herbs or rarely shrubs, sometimes aquatic, leaves alternate, opposite, simple, stipulate, spicate or racemose, often solitary, flowers bisexual. Hypanthium present. Calyx 2-(4)5, corolla 2(4)-5, fruit capsule, nut or berry. Chief genera of the family are *Epilobium*, *Ludwigia*, *Clarkia* and *Oenothera*. It is represented in Pakistan by 4 genera (Hoch & Raven, 1981).

Pollen morphology of the family has been examined by Erdtman (1952), Mitroiu (1963), Ting (1966), Brown (1967), Skvarla *et al.*, (1976, 1978), Moore *et al.*, (1991), Keri & Zetter (1992), Praglowski *et al.*, (1988, 1994) and Rowley & Claugher (1996). Punt *et al.*, (2003) studied pollen morphology of the family Onagraceae from North West Europe. Skvarla *et al.*, (2008) examined pollen morphology of *Epilobium luteum* of the family Onagraceae belonging to tribe Onagreae. Anatomical and pollen morphological characters of the genus *Epilobium* from North West Anatolia have been examined by Makbul *et al.*, (2008). Pocknall & Jarzen (2009) studied the pollen with viscin threads from the late Cretaceous and Paleocene. There are no reports on pollen morphology of the family Onagraceae from Pakistan. Present investigations are based on the pollen morphological studies of 13 species

representing 3 genera of the family Onagraceae by light and scanning electron microscope.

## Materials and Methods

Polleniferous material was obtained from the specimens of Karachi University Herbarium (KUH). 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), equatorial diameter (E), aperture size and exine thickness were measured (Tables 1-3).

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

Table 1. General pollen characters of species found in pollen type-*Circaea alpina*.

Name of species	Pollen length in $\mu\text{m}$	Pollen diameter in $\mu\text{m}$	Exine thickness in $\mu\text{m}$	Tectum
<i>Circaea alpina</i>	19.41(41.11)22.77	1.01(41.12)1.54	2.01(10.1)2.5	scabrate
<i>Circaea cordatan</i> Royle	17.28(18.25)18.36	1.62(2.05)2.16	2.16(2.51)2.71	Sparsely scabrate

Table 2. General pollen characters of species found in pollen type-*Epilobium angustifolium*.

Name of species	Polar length in $\mu\text{m}$	Equatorial diameter in $\mu\text{m}$	Exine thickness in $\mu\text{m}$	Tectum
<i>Epilobium angustifolium</i> L.	71.0(72.11)74.11	60.11(61.8)62.20	9.01(10.1)11.25	fossulate
<i>Epilobium cylindricum</i> D.Don.	71.00(72.25)76.00	70.11(72.1)75.0	2.16(2.51)2.71	Sparsely scabrate
<i>Epilobium chitralense</i> Raven	100.1(103)108.2	90.11(92.11)94.1	2.16(2.51)2.71	Fossulate
<i>Epilobium hitsutum</i> L.	63.22(68.22)68.99	90.22(92.7)94.41	c. 5	Granulate
<i>Epilobium latifolium</i> L.	70.22(72.1)75.11	89.22(92.7)96.12	3.12 (4.51)2.71	Scabrate-subpsilate
<i>Epilobium leiophyllum</i> Hausskn.	40.0(41.2)45.11	60.12(61.8)64.12	4.11(5.51)5.71	Sub-psilate-scabrate
<i>Epilobium minutiflorum</i> Hausskn.	40.22(41.2)45.66	40.12(42.7)45.12	2.14(2.81)2.71	Scabrate-sub-psilate
<i>Epilobium parviflorum</i> Schreber	80.22(82.4)84.11	91.12(92.7)94.12	5.15	Scabrate-subpsilate
<i>Epilobium palustre</i> L.	64.6(68.12)69.12	86.14(89.5)90.22	4.9	Granulate

**Table 3. General pollen characters of species found in pollen type-*Oenothera affinis*.**

Name of species	Pollen diameter in $\mu\text{m}$	Pollen diameter in $\mu\text{m}$	Exine thickness in $\mu\text{m}$	Tectum
<i>Oenothera affinis</i> Cambess.	181.1(185)189.1	142.1(144.2)149.4	5.01(8.5.1)12.5	Scabrate-fossulate
<i>Oenothera glaziovian</i> Michell.	192.8(195)198.3	202.2(206.1)210.0	5.16(8.01)11.71	Fossulate-scabrate

**General pollen characters of the family Onagraceae**

Pollen grains usually tetrads or monads, radially symmetrical, isopolar. Shape mostly oblate. rarely spheroidal. Pollen are 3-zonoporate often 4-zonoporate, apertural membrane granulate, sexine thicker than nexine

rarely thinner than nexine. Tectal scabrate-granulated or striate-rugulate, fossulate-rugulate with viscin threads in most of the species. On the basis of size and tectum types three distinct pollen types are recognized viz., *Circaea alpina*-type *Epilobium angustifolium*-type and *Oenothera affinis*-type.

**Key to the pollen types**

1. + Pollen grains 195  $\mu\text{m}$  ..... *Oenothera affinis*-type
- Pollen grains less than 195  $\mu\text{m}$  ..... 2
2. + Exine ornamentation finely fossulate-rugulate ..... *Circaea alpina*-type
- Exine ornamentation scabrate-sub-psilate rarely striate ..... *Epilobium angustifolium*-type

**Pollen type: *Circaea alpina*-type****Pollen class:** 3-zonoporate**P/E ratio:** 1.00**Shape:** Spheroidal**Apertures:** Porate**Exine:** Sexine thicker than nexine**Ornamentation:** Finely rugulate

**Measurements: Size:** P = (40.12-) 70.2  $\pm$  0.12 (-100)  $\mu\text{m}$ , and breadth (40.1) 68.0  $\pm$  0.9) 96.11)  $\mu\text{m}$ , pore with costae (25.95-) 30.60  $\pm$  1.23 (35.26)  $\mu\text{m}$  in diameter. Mesopodium 42-52  $\mu\text{m}$ . Apopodium 30.1(33.8  $\pm$  1.27) 37.5  $\mu\text{m}$ . Exine 9.0 (9.75  $\pm$  0.15) 10.51  $\mu\text{m}$  thick, sexine thicker than nexine. Tectum finely rugulate with viscin threads.

**Species included:** *Circaea alpina*, *Circaea cordata* Royle**Pollen type: *Epilobium angustifolium*-type (Fig. 1A -F; Fig.2 A-D)****Pollen class:** 3-zonoporate**P/E ratio:** 1.01**Shape:** Spheroidal and suboblate and oblate**Apertures:** More or less circular**Exine:** Sexine thicker than nexine.

**Ornamentation:** Various type densely subsilate or granulated-scabrate, fossulate-rugulate and finely striate with viscin threads.

**Measurements: Size:** Length = (41.2-) 56.97  $\pm$  0.31 (-72.7)  $\mu\text{m}$  and breadth (61.8) 76.90  $\pm$  0.27 (92.01)  $\mu\text{m}$ , Pore with costae c. 359  $\mu\text{m}$  diameter. Mesopodium 41-92, Apopodium 36-61.8  $\mu\text{m}$ . Exine 2.1 (6.65  $\pm$  0.48) 11.1  $\mu\text{m}$  thick, sexine thicker than nexine. Tectum subsilate-finely striate or scabrate-granulate.

**Species included:** *Epilobium angustifolium* L., *E. cylindricum* D. Don., *E. chitralensis* Raven, *E. hirsutum* L. *E. latifolium* L., *E. leiophyllum* Haussn., *E. parviflorum* Schreber

**Pollen type: *Oenothera affinis*-type (Fig. 2E-F).****Pollen class:** 3-zonoporate**P/E ratio:** 100**Shape:** Spheroidal**Apertures:** More or less circular**Exine:** Sexine thicker than nexine**Ornamentation:** Fossulate-rugulate

**Measurements: Size:** Length = (181-) 190.1  $\pm$  2.31 (-195)  $\mu\text{m}$  and breadth (142) 175. 2  $\pm$  0.27 (210)  $\mu\text{m}$ , Pore c. 30.9 long 10-15 broad. Mesopodium 103  $\mu\text{m}$ , Apopodium 41.2  $\mu\text{m}$ . Exine 5.87 (8.56  $\pm$  0.48) 11.25  $\mu\text{m}$  thick, sexine thicker than nexine. Tectum fossulate-rugulate.

**Species included:** *Oenothera affinis* Combess., and *O. glazioviana* Micheli.**Discussion**

Onagraceae pollen are easily recognized from most of the angiosperms families by having tetrads pollen with viscin threads. According to fossil record the family seems to date back to the upper cretaceous (Eyde & Morgan 1973, Skvarla *et al.*, 1978; Martin, 2003). Morphology and molecular analysis suggested that the Onagraceae are monophyletic, with the genus *Ludwigia* as a basal group (Levin *et al.*, 2003). The diverse fossil Onagraceae pollen from a Miocene palynoflora contains the earliest records of *Circaea* pollen in the northern hemisphere, and the earliest fossil record of *Epilobium* pollen from China (Grimsson *et al.*, 2012).

Present pollen data is based on pollen morphology of 13 species representing three genera viz., *Circaea*, *Epilobium* and *Oenothera*. Pollen shows a considerable variation particularly in exine ornamentation and pollen size. On the basis of pollen size and exine pattern three different pollen types are recognized. *Epilobium angustifolium*-type, *Circaea alpina*-type and *Oenothera affinis*-type. Punt *et al.*, (2003) divided the northwest European species of the family Onagraceae into 7 pollen types, division is based on the aperture, size, and exine thickness viz., *Circaea lutetiana*-type, *Epilobium angustifolium*-type, *Epilobium latifolium*-type, *Epilobium tetragonum*-type, *Fuchsia magellanica*-type, *Ludwigia palustris*-type and *Oenothera biennis*-types.

Pollen grains usually radially symmetrical, isopolar, trizonoporate, pollen generally sheds in tetrads rarely monads. In *Epilobium cylindricum* D. Don., *E. chitralensis* Raven, *E. latifolium* L., *E. leiophyllum* Haussn, Royle, *E. parviflorum* Schreber species shed pollen in tetrads whereas, *Circaea alpina*, *Circaea cordata* Royle, *Oenothera affinis* Combess. and *O. glazioviana* Michel., have monads. However, *Epilobium angustifolium* L., and *E. hirsutum* L., both monads and tetrads pollen are found. Shape is sub-oblate to oblate-spheroidal.

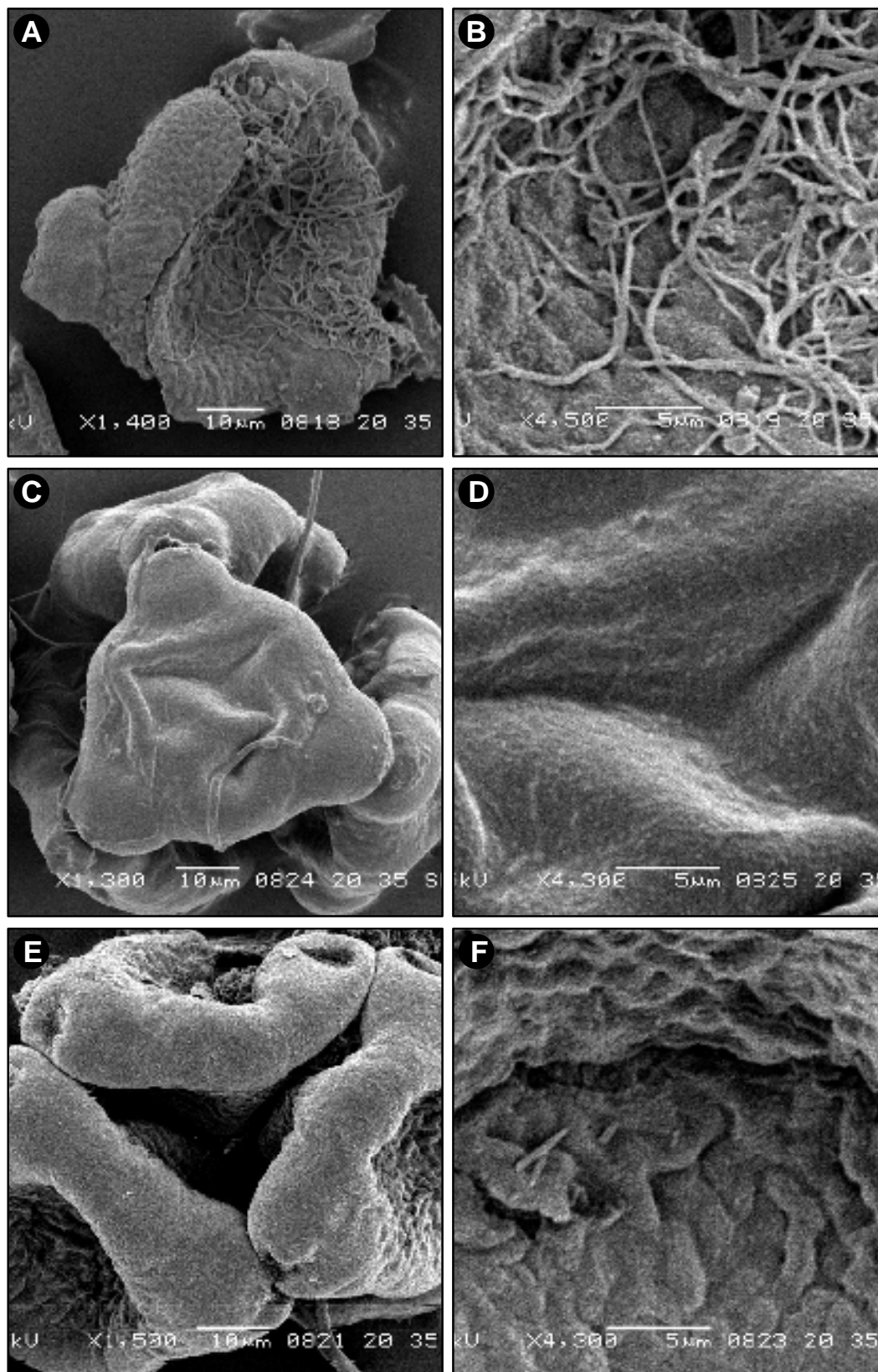


Fig. 1. Scanning electron micrographs of pollen grains: *Epilobium angustifolium*: A. Polar view, B. Exine pattern, *Epilobium cylindricum*: C. Pollen in tetrads, D. Exine pattern, *Epilobium chitralensis*: E. Pollen in tetrads, F. Exine pattern. Scale bar = A, C & E = 10 μm; B, D & F = 5 μm

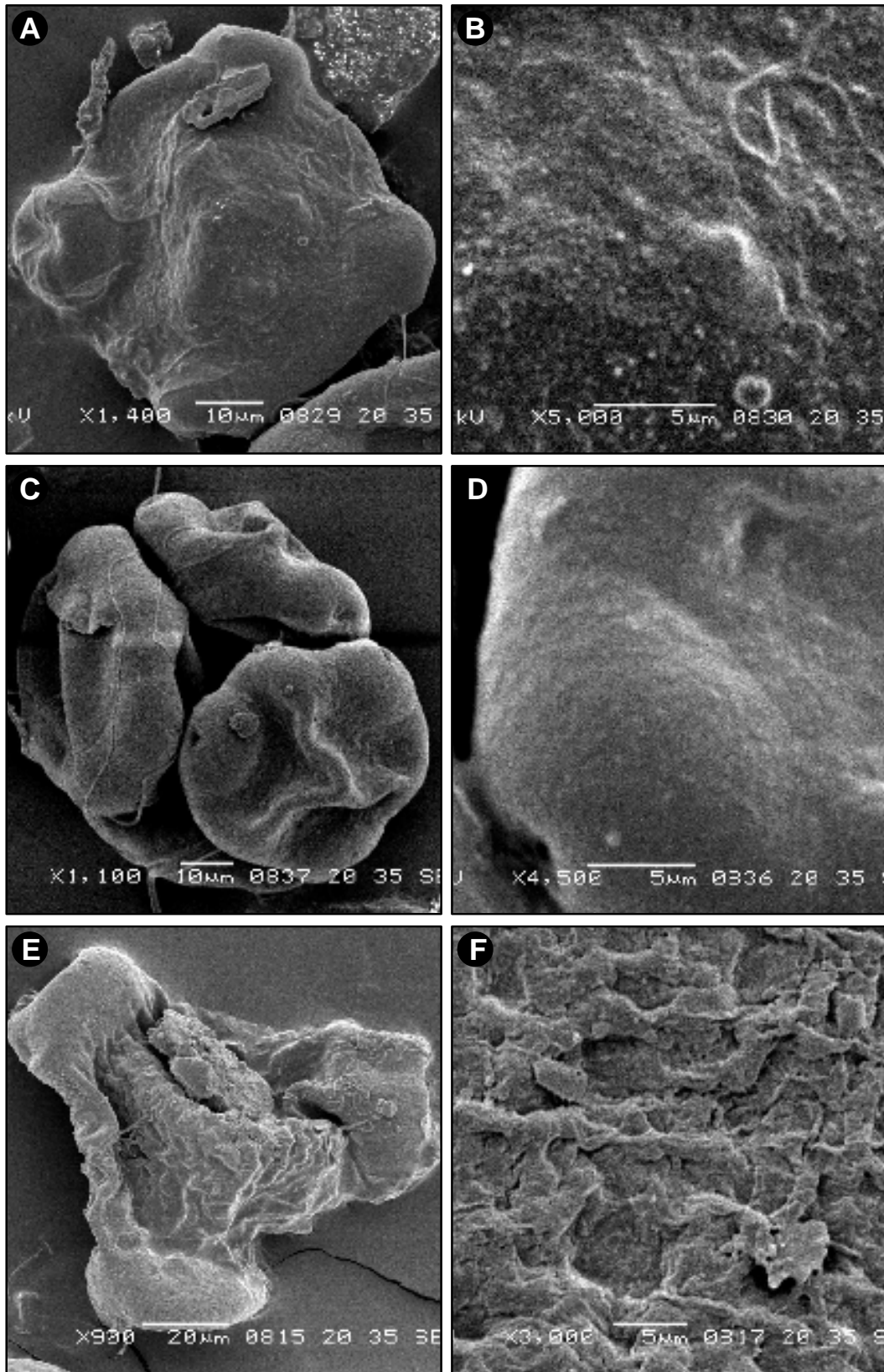


Fig. 2. Scanning electron micrographs of pollen grains: *Epilobium latifolium*: A. Polar view, B. Exine pattern, *Epilobium leiophyllum*: C. Pollen in tetrads, D. Exine pattern, *Oenothera affinis*: E. Polar view, F. Exine pattern. Scale bar = A, C & E = 10 μm; B, D & F = 5 μm

Tectum of the pollen is also significantly variable. For instance, in two species of the genus *Oenothera* (*Oenothera affinis* Combess., and *O. glazioviana* Michel.) tectum is fossulate-rugulate. *Epilobium leiophyllum* Haussn. has granulated exine pattern, whereas, remaining species i.e., *Epilobium angustifolium* E., *cylindricum* D. Don., *E. hirsutum* L., *E. latifolium* L., *E. parviflorum* Schreber, and *E. pulastris* have scabrate tectum. However, *Epilobium chitralenses* is easily recognized by having rugulate to fossulate exine ornamentation. Makbul *et al.*, (2008), reported bacculate tectum in the *Epilobium hirsutum* and *Epilobium palustris*, they also suggested that palynology shows close relationships among *Epilobium* species.

The present palynological investigations is quite helpful the generic level as all the three genera viz., *Circaea*, *Epilobium* and *Oenothera* fall in three different pollen types. However, pollen types *Epilobium angustifolium* and *Circaea alpina*-type Levin, *et al.*, 2003, who have also suggested that the Onagraceae pollen were easily recognized by having tetrads with viscin threads. However, pollen morphology is not helpful at species level.

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