

POLLEN MORPHOLOGY OF THE FAMILY CRASSULACEAE FROM PAKISTAN AND KASHMIR AND ITS TAXONOMIC IMPLICATIONS

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Abstract

Pollen morphology of 28 species representing 9 genera viz., *Bryophyllum*, *Kalanchoe*, *Pseudosedum*, *Rhodiola*, *Rosularia*, *Hylotelephium*, *Sedum*, *Tillaea* and *Orostachys* of the family Crassulaceae from Pakistan has been studied by light and scanning electron microscope. Pollen grains are usually free, radially symmetrical, isopolar, sub-oblate to oblate-spheroidal, or prolate-spheroidal to subprolate often prolate and tricolporate. Tectum mostly striate-rugulate or sub-pilulate rarely reticulate-rugulate. Significant variation is found in shape and exine ornamentation and on the basis these characters family has been divided into three pollen types viz., *Rhodiola sinuata*-type, *Rosularia viguieri*-type and *Sedum hispanicum*-type. Numerical analysis of based on pollen characters of 28 species was also carried out to determine the phylogenetic relationships of various genera and the species of the family Crassulaceae.

Key words: Crassulaceae, Eurapalynous, Exine ornamentation and Pollen shape.

Introduction

Crassulaceae, a family of about 33 genera and c. 1500 species is distributed all over the world, more predominantly in the temperate and subtropical zones of the northern hemisphere and Africa (Mabberley, 1987). Represented in Pakistan by 9 genera and 32 species (Sarwar, 2002). According to Berger (1930) Crassulaceae is a morphologically diverse and systematically complex angiospermic family comprising 35 genera and 1500 species which is usually characterized by succulent habit, follicle fruit, numerous and very small seed. Berger (1930) recognized six subfamilies within the family Crassulaceae viz., Crassuloideae, Cotyledonoideae, Kalanchoideae, Echeverioideae, Sedoideae, and Sempervivoideae on the basis of floral morphological characters such as Crassuloideae include species having a single whorl of stamens (haplostemonous) and free petals, whereas Cotyledonoideae and Kalanchoideae include diplostemonous species with fused corollas. Sempervivoideae all species possess unfused, polymeric flowers, and the Echeverioideae have typically five-merous corollas which are partially to completely fused. Thorne (1983) recognized three subfamilies in Crassulaceae viz., Crassuloideae, Kalanchoideae, and Sedoideae, whereas Van Ham & t Hart (1998) recognized two subfamilies Crassuloideae and Sedoideae sensu lato.

Pollen morphology of the family Crassulaceae has been examined by Nair (1962), Faegri & Iversen (1964), t'Hart (1975), Lee (1978), Moore & Webb (1978), Calie (1981), Hideux (1981), Nyffeler (1992), Oybak *et al.* (1997) and Karaer *et al.* (2010).

Parnell (1991) studied pollen morphology of *Jovibarba Opiz* and *Sempervivum* L. Kim (1994) examined the pollen morphology of the genus *Sedum* from Korea. Phylogenetic relationships in the Crassulaceae based on Chloroplast DNA restriction site variation have been studied by Van Ham & t'Hart, (1998). Mort *et al.* (2001) studied the phylogenetic and evolutionary relationship of family Crassulaceae on matK sequence data. Sin *et al.* (2002) conducted palynotaxonomic study of the Korean Crassulaceae. Lee & J-Hong, (2010) determined the intraspecific sequence variation of trnL/F

intergenic region (cpDNA) in *Sedum* (Crassulaceae) and geographic distribution. There are no reports on the pollen morphology of the family Crassulaceae from Pakistan and Kashmir. However, Numerical taxonomy of the *Sedum* and *Rosularia* from Pakistan has been studied by Sarwar & Qaiser (2011, 2012) respectively. Present study is the first study of pollen morphology of the family Crassulaceae from Pakistan and Kashmir by scanning and light microscope.

Material and Methods

Polleniferous material was obtained from the specimens of Karachi University Herbarium (KUH). In few cases fresh material was also collected from the field. The list of voucher specimens is deposited in KUH (Centre for Plant Conservation). 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 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 were suspended in a drop of water and 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). The coating was restricted to 150 A° and S.E.M examination was carried out on a Jeol microscope JSM-6820. 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 1-2).

Numerical analysis: A Hierarchical cluster analysis of 28 taxa of the family Crassulaceae was carried. The numerical analysis was performed by using the Euclidean distance index and group average strategy with the computer package (SPSS Inc., 1998). Each taxon was treated as operational taxonomic unit (OTU) (Table 3).

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

Result and Observations

General pollen morphology of the family Crassulaceae: Pollen grains usually radially symmetrical, isopolar, sub-oblate to oblate-spheroidal or sub-prolate to prolate-spheroidal, prolate, tricolporate, amb trilobed or triangular, with apertures on the angles of the outline of the grain in polar view,

colpal membrane generally scabrate or granulated, ora circular to transversely elliptic, sexine thicker or thinner than nexine, or as thick as nexine. Tectum generally striate-rugulate, or subpsilate rarely reticulate-rugulate. On the basis of exine surface family has been divided into three pollen types viz., *Rhodiola sinuata*-type, *Rosularia viguieri*-type and *Sedum hispanicum*-type.

Key to the pollen types

1. + Tectum reticulate-rugulate *Rhodiola sinuata*-type
 - Tectum not as above 2
 2. + Tectum subpsilate *Rosularia viguieri*-type
 - Tectum striate-rugulate *Sedum hispanicum*-type

Pollen type -I: *Rhodiola sinuata*

Pollen class: 3-colporate, 3-zonocolporate.

P/E ratio: 0.95

Aperture: Ectoaperture-colpus long, sunken, narrow, end acute. Endoaperture circular

Exine: Sexine thicker than nexine or than nexine.

Ornamentation: Reticulate-rugulate

Outline: Equatorial view elliptic, polar view triangular.

Measurements: Polar axis (18.81-) 22.5 (-26.26) μm , and Equatorial diameter E(18.5-) 20.05 (-23.4) μm ; colpi (15.35-) 21.72 (-28.10) μm in length. Mesocolpium (17.6-) 17.90 (-18.2) μm . Apocolpium (10.40-) 11.05 (-11.7) μm . Exine (1.21-) 1.21 (-1.22) μm .

Species included: *Rhodiola sinuata* (Royle ex Edgew.) S.H.Fu

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

Apertures: Ectoapertures-colpus long, sunken, narrow, margins, Endoaperture circular

Exine: Sexine thicker or thinner than nexine.

Ornamentation: Tectum subpsilate

Outline: Equatorial view elliptic, Polar view trilobed with aperture on the angles of the outline of the grains in polar view.

Measurements: Polar axis P (9.60) 23.45 (27.7) μm . Equatorial diameter (12.4) 20.26 (28.12) μm . Colpus length (8.41) 16.95 (25.50) μm . Mesocolpium (7.00) 14.21 (21.5) μm . Apocolpium 0.75-5.55 μm . Exine (0.25) 1.75 (3.25) μm thick.

Species included: *Rosularia adenotricha* (Wall. ex Edgew.) Jansson, *Rosularia sedoides* (Dence.) H. Ohba, *Rosularia viguieri* (Raym. Hamet ex Frod.) G.R. Sarwar, *Rhodiola quadrifida* (Pallas) Schrenk, *Rhodiola recticaulis* Boriss., and *Rhodiola coccinea* (Royle) Boriss., and *Tillaea schimperii* (C.A.Mey) M.G.Gilbert, H.Ohba & K.T.Fu.

Pollen type-II: *Rosularia viguieri*-type (Fig. 1A & B; Fig. 3 E&F)

Pollen class: 3-colporate, 3-zonocolporate.

P/E ratio: 0.78-1.11

Key to the species of pollen type-II: *Rosularia viguieri*

1. + Pollen grains prolate-spheroidal 2
 - Pollen grains not as above 3
 2. + Polar length 21-26.11 μm *Rosularia sedoides*
 - Polar length c. 28.93 μm *Rosularia viguieri*
 3. + Pollen oblate-spheroidal 4
 - Pollen sub-oblate 5
 4. + Polar length 9.6-14.1 μm *Tillaea schimperii*
 - Polar length c. 15.8 μm *Rhodiola quadrifida* & *Rhodiola recticaulis*
 5. + Exine thickness c. 2.65 μm *Rhodiola coccinea*
 - Exine thickness c. 2.05 μm *Rosularia adenotricha*

Pollen type-III: *Sedum hispanicum* (Fig. 1 C-F; Fig. 2A-F; Fig. 3A-D)

Pollen class: 3-colporate, 3-zonocolporate.

P/E ratio: 0.90-1.48

Shape: Sub-prolate to prolate or oblate-spheroidal

Apertures: Ectoapertures-colpus long, sunken, narrow, margins, Endoaperture circular

Exine: Sexine thicker or thinner than nexine.

Ornamentation: Tectum striate-rugulate

Outline: Equatorial view elliptic, Polar view trilobed with aperture on the angles of the outline of the grains in polar view.

Measurements: Polar axis P (13.3) 20.50 (26.7) μm . Equatorial diameter (13.40) 21.25; (29.10) μm . Colpus length (13.5) 21.00 (28.5) μm . Mesocolpium (10.00) 13.50; (17.00) μm . Apocolpium 0.75-5.55 μm . Exine (0.26) 1.44 (2.63) μm thick.

Species included: *Bryophyllum daigremontianum* (Raym.-Hamet & H. Perrier) A. Berger, *Bryophyllum pinnatum* (Lam.) Oken, *Hylotelephium ewersii* (Ledeb.) H. Ohba, *Hylotelephium pakistanicum* (G.R. Sarwar) G.R. Sarwar, *Kalanchoe integra* (Medik.) Kuntze, *Rosularia alpestris* (Kar. & Kir) Boriss, *Rhodiola bupleuroides* (Wall. ex Hook.f. & Thomson) S.H. Fu, *Rhodiola fastigiata* (Hook.f. & Thomson) S.H. Fu, *Rhodiola heterodonta* (Hook.f. & Thomson) Boriss, *Rhodiola saxifragoides* (Frod.) H. Ohba, *Rhodiola tibetica* (Hook.f.

Thomson) S.H. Fu, *Rhodiola wallichiana* (Hook.f.) S.H. Fu, *Pseudosedum lievenii* (Ledeb.) A. Berger, *P. condensatum* Boriss., *Orostachys thyrsoiflora* Fisch., *Sedum fischeri* Raym.- Hamet, *Sedum hispanicum* L., *Sedum multicaule* Wall. ex Lindl., *Sedum oreades* (Dence.) Raym.-Hamet, *Sedum trullipetalum* Hook.f. & Thomson.

This pollen type is further divided in 5 subgroups based on the shape of pollen grains.

Key to the subgroups of pollen type III: *Sedum hispanicum*

1. + Pollen grains prolate subgroup I (*Rhodiola tibetica*)
- Pollen grains not as above 2
2. + Pollen sub-prolate to prolate-spheroidal 3
- Pollen sub-oblate to oblate-spheroidal 4
3. + Pollen sub-prolate subgroup-II
(*Hylotelephium ewersii* (Ledeb.) H. Ohba, *Hylotelephium pakistanicum* (G.R. Sarwar), *Rhodiola fastigiata* (Hook.f. & Thomson) S.H. Fu, *Rhodiola heterodonta* (Hook.f. & Thomson) Boriss. and *Rhodiola wallichiana* (Hook.f.) S.H. Fu.)
- Pollen prolate-spheroidal subgroup-III
(*Bryophyllum daigremontianum* (Raym.-Hamet & H. Perrier) A. Berger, *Bryophyllum pinnatum* and *Rhodiola bupleuroides* (Wall. ex Hook.f. & Thomson) S.H. Fu.)
4. + Pollen grain sub-oblate subgroup-IV
(*Kalanchoe integra*, *Sedum multicaule* Wall. ex Lindl., and *Sedum trullipetalum* Hook.f. & Thomson.)
- Pollen oblate-spheroidal subgroup-V
(*Sedum hispanicum* L., *Rosularia alpestris* (Kar. & Kir) Boriss, *Rhodiola saxifragoides* (Frod.) H. Ohba, *Orostachys thyrsoiflora* Fisch., *Pseudosedum lievenii* (Ledeb.) A. Berger, *P. condensatum* Boriss., *Sedum fischeri* Raym.-Hamet and *Sedum oreades* (Decne.) Raym.-Hamet).

Result and Discussion

Crassulaceae is a medium size family. It is probably the most cytologically complex angiospermic family, exhibits highly complex cytological and chromosomal variation (Uhl, 1948). Similarly, pollen morphology of the family is also quite heterogeneous. Pollen grains are radially symmetrical, isopolar, suboblate to oblate-spheroidal, prolate-spheroidal or subprolate rarely prolate, tricolporate, trizoncolporate. Generally triangular, trilobed or circular in polar view and elliptic or broadly elliptic in equatorial view. Sexine thicker than or as thick as nexine. Tectum striate-rugulate to psilate rarely reticulate-rugulate. Erdtman (1952) also reported similar pollen in the family Crassulaceae. Hideux (1981) reported tricolporate, sub-spheroidal to prolate pollen with striate, reticulate and rugulate tectum in the family Crassulaceae. Present pollen data is based on pollen morphology of 28 species representing 9 genera viz., *Bryophyllum*, *Kalanchoe*, *Pseudosedum*, *Rhodiola*, *Rosularia*, *Hylotelephium*, *Sedum*, *Tillaea* and *Orostachys*. Pollen shows a considerable variation particularly in exine ornamentation and pollen shape class. On the basis of exine ornamentation three distinct pollen types are recognized viz., *Rosularia viguieri*-type, *Rhodiola sinuata*-type and *Sedum hispanicum*-type.

Pollen type-I: *Rhodiola sinuata*

It is characterized by reticulate-rugulate tectum. Pollen grains oblate-spheroidal, tricolporate, sexine as thick as nexine. Tectum reticulate-rugulate. It comprises only one species i.e., *Rhodiola sinuata* Royle ex Edgew.) S.H. Fu

Pollen type-II: *Rosularia viguieri*

This pollen type is easily delimited by subsilate tectum. Pollen grains sub-prolate, oblate-spheroidal or

sub-oblate, tricolporate, sexine thicker than nexine or as thick as nexine. Represented by 7 taxa viz., *Rosularia sedoides* (Dence.) H. Ohba, *Rosularia viguieri* (Raym. Hamet ex Frod.) G.R. Sarwar, *Rhodiola quadrifida* (Pallas) Schrenk, *Rhodiola recticaulis* Boriss., *Rosularia adenotricha* (Wall. ex Edgew.) Jansson and *Rhodiola coccinea* (Royle) Boriss. and *Tillaea schimperii* (C.A. Mey) M.G. Gilbert, H. Ohba & K.T. Fu

Pollen type-III: *Sedum hispanicum*

It is characterized by striate-rugulate tectum. Pollen grains oblate-spheroidal to prolate-spheroidal, or sub-prolate rarely suboblate or prolate, tricolporate, sexine thicker than nexine or as thick as nexine. Tectum striate-rugulate. It is the largest pollen type and accommodates 20 taxa belonging to all the studied genera, representing 74.07% of the total taxa and includes such as, *Bryophyllum daigremontianum* (Raym.-Hamet & H. Perrier) A. Berger, *Bryophyllum pinnatum* (Lam.) Oken, *Hylotelephium ewersii* (Ledeb.) H. Ohba, *Hylotelephium pakistanicum* (G.R. Sarwar) G.R. Sarwar, *Kalanchoe integra* (Medik.) Kuntze, *Sedum fischeri* Raym.- Hamet, *Sedum hispanicum* L., *Sedum multicaule* Wall. ex Lindl., *Sedum oreades* (Dence.) Raym.-Hamet, *Sedum trullipetalum* Hook.f. Thomson, *Rosularia alpestris* (Kar. & Kir) Boriss, *Rhodiola bupleuroides* (Wall. ex Hook.f. & Thomson) S.H. Fu, *Rhodiola fastigiata* (Hook.f. & Thomson) S.H. Fu, *Rhodiola heterodonta* (Hook.f. & Thomson) Boriss, *Rhodiola saxifragoides* (Frod.) H. Ohba, *Rhodiola tibetica* (Hook.f. Thomson) S.H. Fu, *Rhodiola wallichiana* (Hook.) S.H. Fu, *Pseudosedum lievenii* (Ledeb.) A. Berger, *P. condensatum* Boriss., *Orostachys thyrsoiflora* Fisch. This pollen type is further subdivided in to five groups on the basis of pollen shape.

Table 1. General pollen characters found in pollen type *Rosularia viguieri*.

Name of species	Polar axis (P) in μm	Equatorial diameter in μm	Shape	Colpus length μm	Mesocolpium μm	Apocolpium μm	Exine thickness
<i>Rodiola quadrifida</i> (Pallas) Schrenk	15.58	15.78	Ob-sp	10.52	13.5	7.84	2.63
<i>Rosularia sedoides</i> (Dence.) H. Ohba	21.64 (23.67) 26.3	21.014 (23.6) 22.32	Pr-sp	15.78 23.67	15.78	11.83	2.63
<i>Rosularia viguieri</i> (Raym. Hamet ex Frod.) G.R. Sarwar	28.93	26.3	Pr-sp	27.6	15.78	10.00	1.13
<i>Tillaea schimperii</i> (C.A. Mey) M.G. Gilbert, H. Ohba & K.T. Fu.	(9.6) 12(14.1)	(12) 13.2	Ob-sp	(8.4) 10.2	(7.2) 9.6	(4.8) 6	1.2
<i>Rhodiola coccinea</i> (Royle) Boriss.	15.31	(16.86) 18.41	Sub-pr	12.62 (14.06) 15.51	13.41	7.89	2.63
<i>Rhodiola recticaulis</i> Boriss.	15.78(18.41) 21.04	15.78 (21.04)	Ob-sp	15.78 (18.41) 21.04	17.04	7.84	2.08
<i>Rosularia adenotricha</i> (Wall ex Edgew.) Jansson	18.67	20.5	Ob-sp	13.67	(9.6) 10.8	7.62	2.05

Abbreviations: Ob-sp = Oblate-spheroidal, Pr-sp = Prolate-spheroidal, Sub-pr = Sub-prolate, Pr = prolate

Present studies of Crassulaceae revealed that the family has five pollen shape classes i.e., sub-oblate, oblate-spheroidal, prolate-spheroidal, sub-prolate and prolate. Among these oblate-spheroidal is the most frequent one and found in 16 species distributed in five genera such as, *Bryophyllum*, *Sedum*, *Rhodiola*, *Rosularia* and *Tillaea*, whereas prolate subclass is most rare and found only in a single species of the genus *Rhodiola* (*Rhodiola tibetica*).

Tectum of the pollen is also significantly variable. However, more common exine pattern is striate-rugulate, besides this psilate to sub-psilate, rarely reticulate-rugulate types of exine ornamentation are also found within the family.

Crassulaceae is a eurypalynous family at the generic level also. Most of the genera exhibit different pollen characters and can be segregated easily on the basis of combination of various pollen morphological characters. Considerable variation in the ornamentation of the tectum has been observed in the family Crassulaceae. *Rhodiola* is the only genus which has all the three types of tectum i.e., psilate, striate-rugulate and reticulate-rugulate, suggesting the heterogeneity of the genus. *Rhodiola sinuata* is a unique species in the whole genus which has reticulate-rugulate tectum whereas, the remaining species have striate-rugulate or psilate tectum. Similarly, two types of tectum are also observed in the genus *Rosularia* viz., psilate and striate-rugulate.

From the genus *Bryophyllum* two species have been studied (*Bryophyllum pinnatum* (Lam.) Oken, *Bryophyllum daigremontianum* (Raym.-Hamet & H. Perrier) A. Berger), Both the species are easily delimited on the basis of pollen shape, *Bryophyllum pinnatum* has oblate-spheroidal pollen whereas, *B. daigremontianum* has prolate-spheroidal pollen. Similarly, in the genus *Hylotelephium* 2 species have been examined both are separated on the basis of pollen diameter in *Hylotelephium ewersii* (Ledeb) H. Ohba, pollen diameter is more than 16 μm , whereas another species of the same genus (*Hylotelephium pakistanicum* (G.R. Sarwar) G.S. Sarwar) pollen has less than 16 μm in diameter. Within the genus *Sedum* pollen morphology of five species has been determined; all these species show distinct variation in pollen shape class such as, *Sedum multicaule* Wall. ex Lindl., and *Sedum trullipetalum* Hook. f. Thomson, have sub-oblate pollen whereas remaining species i.e., *Sedum fischeri* Raym.-Hamet, *Sedum hispanicum* L and *Sedum oreades* (Dence.) Raym.-Hamet have oblate-spheroidal pollen. However, these species are further delimited on the basis of polar length (Table 2).

Pollen data from the genus *Rosularia* is based on four species and these species are also easily delimited on the basis of exine surface like, *Rosularia adenotricha* (Wall. ex Edgew.) Jansson, *Rosularia sedoides* (Dence.) H. Ohba, *Rosularia viguieri* (Raym. Hamet ex Frod.) G.R. Sarwar is easily recognized by having sub-psilate exine surface, whereas the *Rosularia alpestris* (Kar. & Kir) Boriss., has striate-rugulate exine ornamentation. Meikle (1977) also reported striate-rugulate exine pattern in two endemic species of the genus *Rosularia* viz., *Rosularia cypria* and *Rosularia pallidiflora*, confined to North Cyprus, he suggested that the palynological resemblances among *Rosularia* species are also found in another member of the same family, *Sedum lampusae* and all these species have *Sedum*-type pollen. However, it was observed that *Sedum* pollen were smaller as compared to *Rosularia* pollen. Present data also showed more or less the similar results (Table 2).

Table 2. General pollen characters found in pollen type *Sedum hispanicum*

Name of species	Polar axis (P) in μm	Equatorial diameter in μm	Shape	Colpus length μm	Mesocolpium μm	Apocolpium μm	Exine thickness
<i>Byrophyllum daigremontianum</i> (Raym.-Hamet & H. Perrier) A. Berger	21.00 (24.98) 23.1	22.11 (23.67) 24.00	Pr-Sp	c. 18.41	20-23	5.00	2.10
<i>Bryophyllum pinnatum</i> (Lam.) Oken	(18.81-) 22-5 (-26.26)	(18.5-) 19.65 (-23.4) 22.35	Ob-sp	(15.35-) 21.72 (-28.10)	(17.6-) 17.15 (-18.2)	(10.40-) 10.05 (-11.7)	(91.21-) 1.21 (-1.22)
<i>Hylotelephium eversii</i> (Ledeb.) H. Ohba	14.43 (23.95) 17.09	17.09 (18.40) 19.72	Sub-pr	13.67 (13.54) 13.41	c. 16.90	7.00	2.00
<i>Hylotelephium pakistanicum</i> (G.R. Sarwar) G.R. Sarwar	15.51	13.44	Sub-pr	13.15	10.52 (17.09) 13.15	6.21	0.26
<i>Pseudosedum lievenii</i> (Ledeb.) A. Berger	18.41 (19.72) 21.04	14.3 (13.9) 13.15	Ob-sp	10.52 (11.83) 13.15	13.5	6.57	2.35
<i>Orostachys thyrsiflora</i> Fisch.	13.15	13.93	Ob-sp	13.41	12.11 (12.81) 13.00	9.79	2.81
<i>Sedum fischeri</i> Raym.-Hamet	26.3 (28.93) 31.56	21.04 (25.11) 29.19	Ob-sp	23.67 (26.07) 28.48	21.04	8.15	2.60
<i>Sedum hispanicum</i> L.	21.04 (23.67) 26.3	23.69 (24.99) 26.3	Ob-sp	21.04	21.04	7.15	2.63
<i>Rhodiola bupleuroides</i> (Wall. ex Hook.f. & Thomson) S.H. Fu	15.78	14.46 (15.5) 16.56	Pr-sp	13.15	13.15	7.89	2.63
<i>Rhodiola fastigiata</i> (Hook.f. & Thomson) S.H. Fu	15.78 (17.09) 18.41	14.46 (15.12) 15.78	Sub-pr	13.15	17.09	7.89	2.63
<i>Rhodiola heterodonta</i> (Hook.f. & Thomson) Boriss.	17.09	18.10	Sub-pr	13.15 (14.72) 16.30	14.72 (15.25) 15.78	6.34	2.90.
<i>Rhodiola tibetica</i> (Hook.f. Thomson) S.H. Fu	18.41 (19.72) 21.04	14.3 (13.9) 13.15	Pr	10.52 (11.83) 13.15	13.5	6.57	2.35
<i>Rhodiola wallichiana</i> (Hook.) S.H. Fu	18.93 (19.85) 20.77	15.25 (15.51) 15.78	Sub-pr	10.52	12.11 (12.81) 13.00	9.99	2.99
<i>Rosularia alpestris</i> (Kar. & Kir) Boriss.	18.93	21.64	Ob-sp	13.41	12.65 (13.03) 13.14	7.91	2.00
<i>Pseudosedum condensatum</i> Boriss	(14.4) 15.6 (16.8)	(19.2) 21.6	Sub-obo	(14.4) 16.8	(9.6) 10.8	(7.2) 8.4	2.4
<i>Sedum trullipetalum</i> Hook.f. & Thomson	(14.4) 16.8	(21.6) 22.8	Sub-obo	(16.8) 18	(9.6) 10.8	(6) 7.8	2.4
<i>Sedum oreades</i> (Dence.) Raym.-Hamet	21.04	21.04	Ob-sp	18.15	10.52	1.31	2.00
<i>Kalanchoe integra</i> (Medik.) Kuntze	(16.81-) 18.01 (-19.21)	(19.25-) 21.68 (-24.11)	Sub-Obbo	(14.35-) 17.50 (-21.8)	(9.6) 10.90 (-12.2)	(6.11-) 6.66	2.4
<i>Rhodiola saxifragoides</i> (Ford.) H. Ohba	(13.2-) 15.60 (-18.00)	(13.41-) 16.57 (-19.74)	Ob-sp	(10.35-) 11.00 (-13.21)	c. 7.89	c. 6.51	(1.30-) 1.31 (-1.32)
<i>Sedum multicaule</i> Wall. ex Lindl.	(16.8) 18	(21.6) 22.8	Sub-obo	(16.8) 18	(12) 13.2	(7.2) 8.4	2.4

Table 3. Data matrix of the family Crassulaceae scored 21 characters presented in Table 3.

Name of species	1	2	3	4	5	6
<i>Bryophyllum daigremontianum</i>	21.00 (24.98) 23.1	22.11 (23.67) 24.00	c. 18.41	20-23	5.00	2.10
<i>Bryophyllum pinnatum</i>	(18.81-) 22-5 (-26.26)	(18.5-) 19.65 (-23.4) 22.35	(15.35-) 21.72 (-28.10)	(17.6-) 17.15 (-18.2)	(10.40-) 10.05 (-11.7)	(1.21-) 1.21 (-1.22)
<i>Hylotelephium ewersii</i>	14.43 (23.95) 17.09	17.09 (18.40) 19.72	13.67 (13.54) 13.41	c. 16.90	7.00	2.00
<i>Hylotelephium pakistanicum</i>	15.51	13.44	13.15	10.52 (17.09) 13.15	6.21	0.26
<i>Kalanchoe integra</i>	(16.81-) 18.01 (-19.21)	(19.25-) 21.68 (-24.11)	(14.35-) 17.50 (-21.8)	(9.6) 10.90 (-12.2)	(6.11-) 6.66	2.4
<i>Orostachys thyrsiflora</i>	13.15	13.93	13.41	12.11 (12.81) 13.00	9.79	2.81
<i>Pseudosedum condensatum</i>	(14.4) 15.6	(19.2) 21.6	(14.4) 16.8	(9.6) 10.8	(7.2) 8.4	2.4
<i>Pseudosedum lievenii</i>	18.41 (19.72) 21.04	14.3 (13.9) 13.15	10.52 (11.83) 15.15	13.5	6.57	2.35
<i>Rhodiola bupleuroides</i>	15.78	14.46 (15.5) 16.56	13.15	13.15	7.89	2.63
<i>Rhodiola coccinea</i>	15.31	(16.86) 18.41	12.62 (14.06) 15.51	13.41	7.89	2.63
<i>Rhodiola fastigiata</i>	15.78 (17.09) 18.41	14.46 (15.12) 15.78	13.15	17.09	7.89	2.63
<i>Rhodiola heterodonta</i>	17.09	18.10	13.15 (14.72) 16.30	14.72 (15.25) 15.78	6.34	2.90
<i>Rodiola quadrifida</i>	15.58	15.78	10.52	13.5	7.84	2.63
<i>Rhodiola recticaulis</i>	15.78 (18.41) 21.04	15.78 (21.04)	15.78 (18.41) 21.04	17.04	7.84	2.08
<i>Rhodiola saxifragoides</i>	(13.2-) 15.60 (-18.00)	(13.41-) 16.57 (-19.74)	(10.35-) 11.00 (-13.21)	c. 7.89	c. 6.51	(1.30-) 1.31 (-1.32)
<i>Rhodiola sinuata</i>	(18.81-) 22.5 (-26.26)	(18.5-) 19.65 (-23.4) 22.35	(15-35-) 21.72 (-28.10)	(17.6-) 17.15 (-18.2)	(10.40-) 10.05 (-11.7)	(1.21-) 1.21 (-1.22)
<i>Rhodiola tibetica</i>	18.41 (19.72) 21.04	14.3 (13.9) 13.15	10.52 (11.83) 13.15	13.5	6.57	2.35
<i>Rhodiola wallichiana</i>	18.93 (19.85) 20.77	15.25 (15.51) 15.78	10.52	12.11 (12.81) 13.00	9.99	2.99
<i>Rosularia adenotricha</i>	18.67	20.5	13.67	(9.6) 10.8	7.62	2.05
<i>Rosularia alpestris</i>	18.93	21.64	13.41	12.65 (13.03) 13.14	7.91	2.00
<i>Rosularia sedoides</i>	21.64 (23.67) 26.3	21.014 (23.6) 22.32	15.78 23.67	15.78	11.83	2.63
<i>Rosularia vignieri</i>	28.93	26.3	27.6	15.78	10.00	1.13
<i>Sedum fischeri</i>	26.3 (28.93) 31.56	21.04 (25.11) 29.19	23.67 (26.07) 28.48	21.04	8.15	2.60
<i>Sedum hispanicum</i>	21.04 (23.67) 26.3	23.69 (24.99) 26.3	21.04	21.04	7.15	2.63
<i>Sedum multicaule</i>	(16.8) 18	(21.6) 22.8	(16.8) 18	(12) 13.2	(7.2) 8.4	2.4
<i>Sedum oreades</i>	21.04	21.04	18.15	10/52	1.31	2.00
<i>Sedum trullipetalum</i>	(14.4) 16.8	(21.6) 22.8	(16.8) 18	(9.6) 10.8	(6.7) 8	2.4
<i>Tillaea schimperii</i>	(9.6) 12	(12) 13.2	(8.4) 10.2	(7.2) 9.6	(4.8) 6	1.2

Table 3. (Cont'd.)

Name of species	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
<i>Byophyllum daigremontianum</i>	1	0	0	0	1	0	0	1	0	1	0	0	0	1	0
<i>Bryophyllum pinnatum</i>	1	0	0	1	0	0	0	1	0	1	0	0	0	1	0
<i>Hylotelephium ewersii</i>	1	0	0	0	0	1	0	1	0	1	0	0	0	1	0
<i>Hylotelephium pakistanicum</i>	1	0	0	0	0	1	0	1	0	0	1	0	0	1	0
<i>Kalanchoe integra</i>	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0
<i>Orostachys thyrsiflora</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0
<i>Pseudosedum condensatum</i>	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0
<i>Pseudosedum lievenii</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0
<i>Rhodiola bupleuroides</i>	1	0	0	0	1	0	0	1	0	0	0	0	0	1	0
<i>Rhodiola coccinea</i>	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1
<i>Rhodiola fastigiata</i>	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0
<i>Rhodiola heterodonta</i>	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0
<i>Rodiola quadrifida</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1
<i>Rhodiola recticaulis</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1
<i>Rhodiola saxifragoides</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1
<i>Rhodiola sinuata</i>	1	0	0	0	1	0	0	1	0	0	0	0	1	0	0
<i>Rhodiola tibetica</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0
<i>Rhodiola wallichiana</i>	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0
<i>Rosularia adentotricha</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1
<i>Rosularia alpestris</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0
<i>Rosularia sedoides</i>	1	0	0	0	1	0	0	1	0	0	0	0	0	0	1
<i>Rosularia viguieri</i>	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1
<i>Sedum fischeri</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0
<i>Sedum hispanicum</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0
<i>Sedum multicaule</i>	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0
<i>Sedum oreades</i>	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0
<i>Sedum trullipetalum</i>	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0
<i>Tillaea schimperi</i>	1	0	0	1	0	0	0	1	0	0	1	0	0	0	1

Key: 1 Polar axis (p) in μm , 2 Equatorial axis (μm), 3 Colpus length (μm), 4, Mesocolpium (μm), 5 Apocolpium (μm), 6 Exine thickness (μm), 7 Isopolar, 8 Apolar, 9 Sub-oblato, 10 Oblate-spheroidal, 11 Prolate-spheroidal, 12 Sub-prolate, 13 Prolate, 14 Symmetrical, 15 Asymmetrical, 16 Thicker than nexine, 17 Sexine thinner than nexine, 18 Sexine as thick as nexine, 19 Reticulate-rugulate, 20 Striate-rugulate, 21 Psilate-subpsilate

Pollen morphological studies do not support infrageneric classification in the family with the exception of the genus *Rosularia*. All the species of *Rosularia* occurring in Pakistan fall in two sections (Sarwar & Qaiser, 2012). The section *Ornithogalopsis* is characterized by striate-rugulate ornamentation, whereas the section *Sempervivella* is characterized by psilate tectum.

Within the genus *Rhodiola* pollen morphology of ten species have been studied, similar to the genus *Rosularia* this genus also differs in exine surface. *Rhodiola coccinea* (Royle) Boriss. *Rhodiola quadrifida* (Pallas) Schrenk and *Rhodiola recticaulis* have subpsilate exine ornamentation. However, the remaining species viz., *Rhodiola bupleuroides* (Wall. ex Hook.f. & Thomson) S.H. Fu, *Rhodiola fastigiata* (Hook.f. & Thomson) S.H. Fu, *Rhodiola heterodonta* (Hook.f. & Thomson) Boriss., *Rhodiola saxifragoides* (Frod.) H. Ohba, *Rhodiola tibetica* (Hook.f. Thomson) S.H. Fu, *Rhodiola wallichiana* (Hook.) S.H. Fu, have striate-rugulate tectum.

Rhodiola sinuata Royle ex Edgew.) S.H. Fu., is delimited by having reticulate-rugulate tectum. However, these species are further delimited on the basis of pollen shape. Remaining genera also have striate-rugulate exine surface viz., *Pseudosedum* (*Pseudosedum lievenii* (Ledeb.) A. Berger) and *Orostachys* (*Orostachys thyrsoiflora* Fisch) are also delimited on pollen shape.

The family has long been considered a natural group (Schonland, 1891; Berger, 1930), and generally considered to be monophyletic and classified in the Ranalean complex (Morgan & Soltis, 1993; Fischerbein *et al.*, 2001), in which they show close affinities with Saxifragaceae (Takhtajan, 1980, Cronquist, 1981, Thorne, 1983), within the complex Crassulaceae are relatively primitive family in floral, structure and embryological features (Takhtajan, 1980).

The cluster analysis of 21 OUTs based on pollen characters of the family Crassulaceae clearly indicates the presence of two major clades.

The clade-1 is comprises of 8 species belonging to five genera viz., *Rhodiola*, *Rosularia*, *Sedum* and *Orostachys* and *Pseudosedum*. This group is usually characterized by the presence of scaly leaves; basal rosette (Sarwar, 2002). However, the genus *Sedum* is recognized by absence of scaly leaves and basal rosette. Pollen morphology, also supports this division because all these species are characterized by having oblate-spheroidal shape and striate-rugulate tectum (fall in *Sedum hispanicum* pollen-type). Clade-1 also showed that closely related taxa may or may not have similar pollen grains. This is clearly depicted in *Sedum hispanicum*, *Sedum fischeri*, *Sedum oreades*, *Pseudosedum condensatum* and *Pseudosedum lievenii*. The first three are most closely allied taxa and the pollen shape and exine thicknesses are similar, which are oblate-spheroidal and 2.4 μm . The other two species are closely related, but have different types of pollen grains. In *Pseudosedum condensatum* pollen grains are suboblate in shape, length 14.4-16.8 breadth 19.2-24 μm and P/E ratio 72.22. While, in *Pseudosedum lievenii* the pollen grains are oblate spheroidal in shape, length 18-21 μm , breadth 14-15 μm

and P/E ratio 94.73. Within the cluster analysis the species of the genus *Pseudosedum* is also distributed in two different clades (Fig. 4). However, the genus *Sedum* shows close affinity with the clade -2. Moreover, the molecular data (van Ham & t' Hart 1998; Mort *et al.*, 2001 and Sarwar & Qaiser, 2012) indicated that the genus *Sedum* was paraphyletic. Furthermore, in the cluster analysis the clade-1 is occupied the basal position, it is also shown that the primitive nature of the genus *Sedum* (out of six species three belong to this clade), the genus *Sedum* has some of the least specialized species groups within the subfamily Sempervivoideae characterized by the basic features of the family, central position of the *Sedum* with regard to the evolution of Crassulaceae has been stressed by t'Hart (1982). Clade 2 in the cluster analysis is the largest group and comprises of eight genera and 20 species. However, this group is further divided into two subgroups A & B. Subgroups A accommodates 7 species distributed in three genera viz., *Rhodiola*, *Rosularia* and *Tillaea*. The genus *Tillaea* is characterized by having connate leaves at the base, minute flowers two seeds per fruit, whereas the remaining genera have numerous seeds per fruit, pollen morphologically also these species belong to pollen type *Rosularia viguier* which is characterized by sub-psilate tectum. However, within this clade the genus *Tillaea* shows separate lineage because it has small pollen among all the studied taxa,

Subgroup B consists of 13 species of genera viz., *Bryophyllum*, *Kalanchoe*, *Pseudosedum*, *Rhodiola*, *Hylotelephium* and *Sedum*. Pollen morphologically all these species have striate-rugulate tectum except *Rhodiola sinuata*, which is easily delimited by having reticulate-rugulate tectum. However, with in the cluster analysis this species shows close relationship to other species of the genus *Rhodiola*. Furthermore, *Bryophyllum*, *Kalanchoe* and *Hylotelephium* show close affinities in the dendrogram based on pollen character, morphologically also these genera are easily distinguished by the absence of scaly and basal rosette leaves and large to small flowers. However, *Bryophyllum* and *Kalanchoe* are more closely related genera than the genus *Hylotelephium* due to the presence of 4-merous flowers, large, branched stem, fused petals and numerous fruit per fruit. Furthermore, Clade-2, subgroup B occupied terminal position which indicates the advancement of the clade-2, because morphologically *Kalanchoe* and *Bryophyllum* are highly derived genera (Thiede & Egli, 2007).

Our results are in agreement with that of Takhtajan (1969, Thorne (1968) who placed the family Crassulaceae within the order Saxifragales next to family Saxifragaceae, as pollen morphology of Crassulaceae is more or less similar to that of Saxifragaceae (Erdtman, 1952,) within the family Saxifragaceae tricolporate pollen with striate-rugulate exine pattern are common (Perveen & Qaiser, 2009).

It is therefore evident from the above discussion that pollen morphological characters are quite important and helpful in delimiting the taxa of Crassulaceae. Our observations are also in agreement with the findings of other workers (t'Hart, 1975 and Egli, (1988)

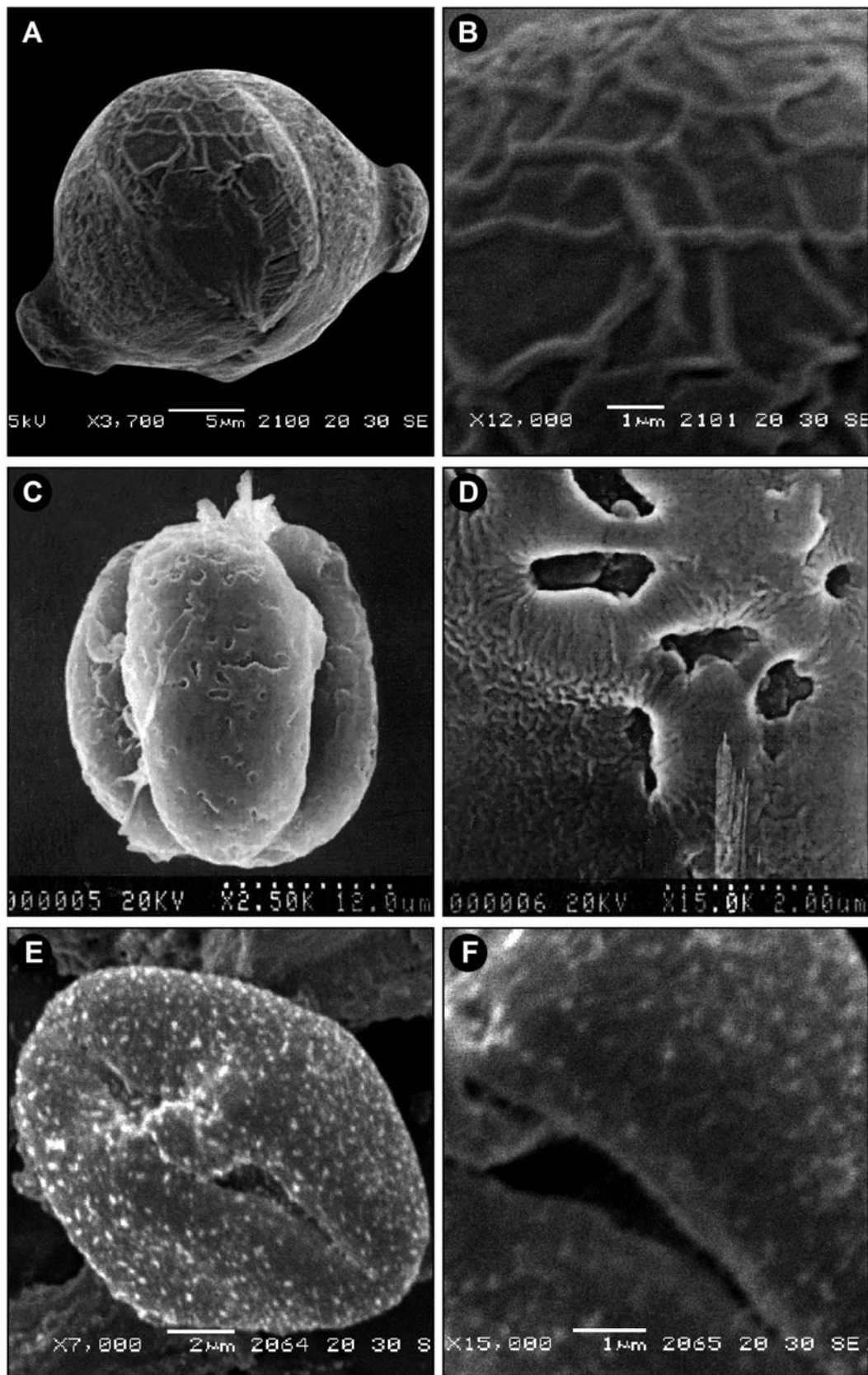


Fig. 1. Scanning electron micrographs (S.E.M.) of the pollen grains: *Bryophyllum pinnatum*: A, Equatorial view, B, Exine pattern. *Kalanchoe integra*: C, Equatorial view; D, Exine pattern. *Rhodiola saxifragoides*: E, Equatorial view; F, Exine pattern.

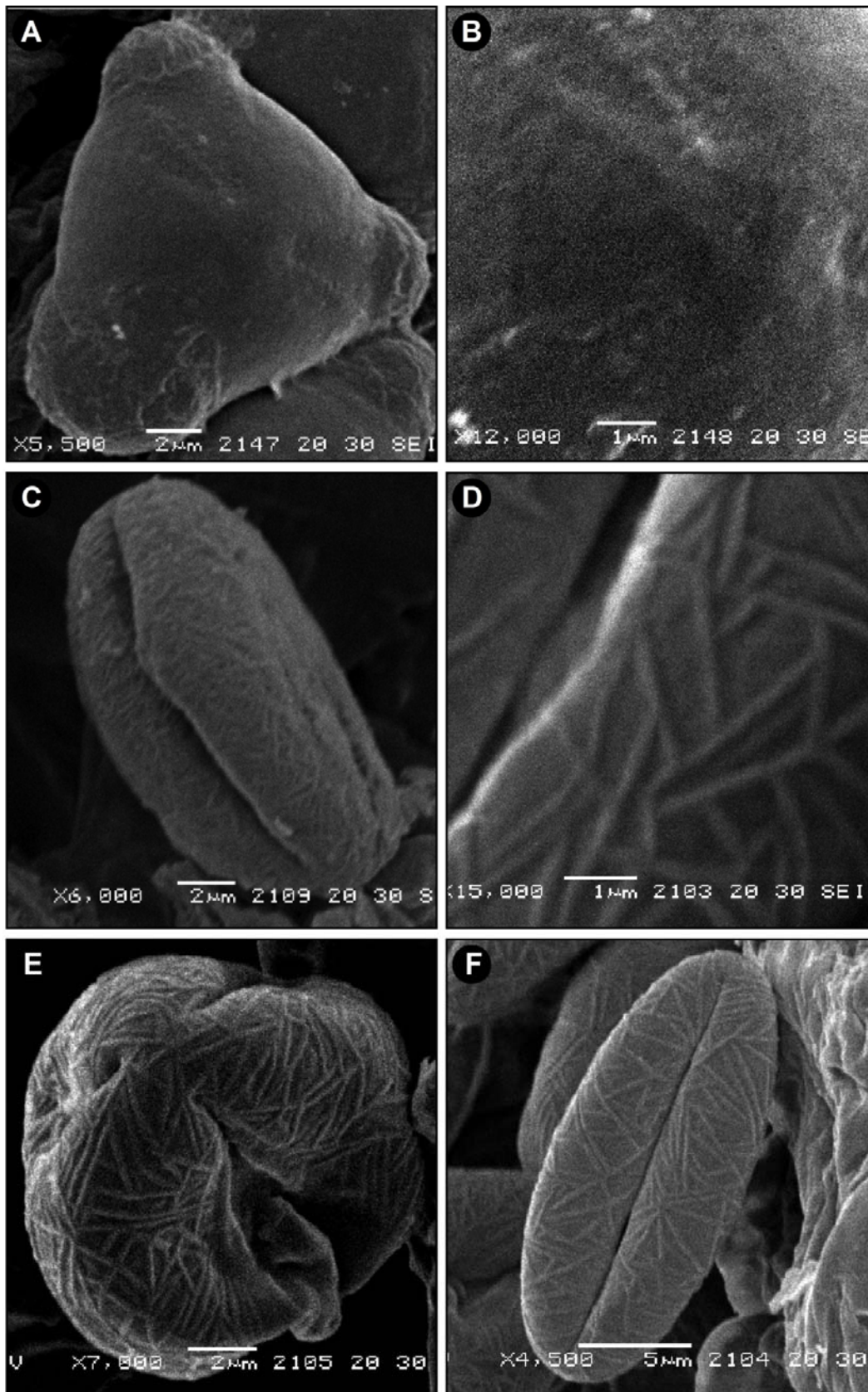


Fig. 2. Scanning electron micrographs (S.E.M.) of the pollen grains: *Rosularia viguieri*: A, polar view, B, Exine surface. *Rhodiola tibetica*: C, Equatorial view, D, Exine pattern. *Hylotelephium ewersii*: E, Polar view, F, Equatorial view.

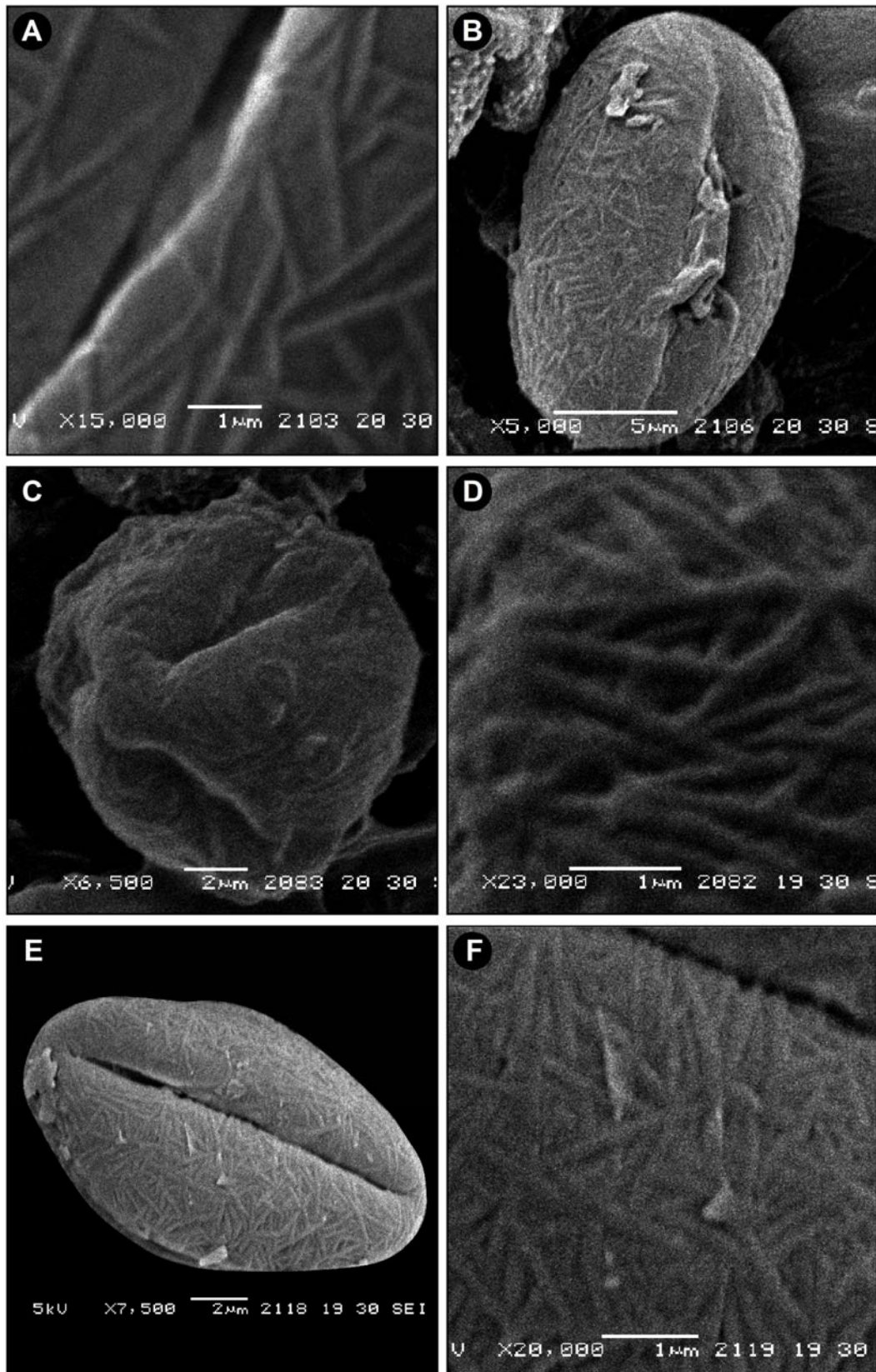


Fig. 3. Scanning electron micrographs (S.E.M.) of the pollen grains: *Hylotelephium ewersii*: A, Exine surface; *Hylotelephium pakistanicum*: B, Equatorial view, *Rhodiola bupleuroides*: C, Polar view, D, Exine pattern. *Rhodiola recticaulis*: E, Equatorial view, F, Exine pattern.

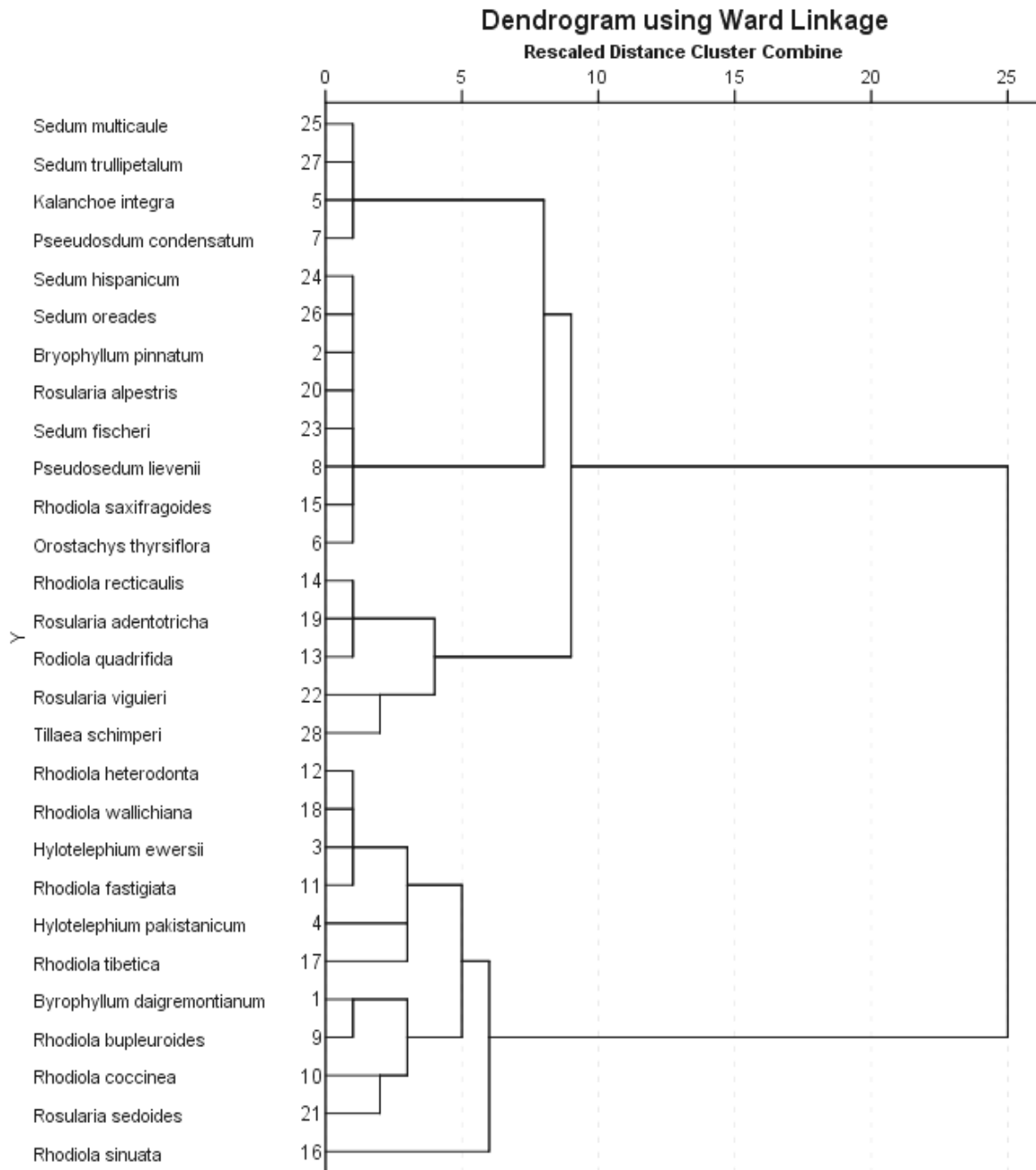


Fig. 4. Dendrogram showing the relationships of the species of the family Crassulaceae.

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