

POLLEN MORPHOLOGY OF THE GENUS *PEDICULARIS* L. OROBANCHACEAE FROM PAKISTAN AND KASHMIR AND ITS TAXONOMIC IMPLICATIONS

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Abstract

Pollen morphology of 22 species of the genus *Pedicularis* L. belonging to 11 series from Pakistan and Kashmir was examined by light and scanning electron microscope. Out of 22 species, pollen of 16 species were examined for the first time, of which 6 species were endemic to the area under consideration. The genus *Pedicularis* is a \pm eurypalynous taxon. Pollen grains are usually radially symmetrical, isopolar, tri-syncolpate and bi-syncolpate, prolate-spheroidal, oblate-spheroidal, sub-prolate rarely prolate. Sexine is slightly thicker than nexine or as thick as nexine. Tectum is quite variable, ranging from psilate to fine-medium scabrate-punctate or micro-foveolate-rugulate. On the basis of exine ornamentation four distinct pollen types viz., *Pedicularis albida*-type, *Pedicularis oederi*-type, *Pedicularis bicornuta*-type, and *Pedicularis roylei*-type were recognized. Little correlation was found between the infrageneric classification and the pollen type. Species belonging to one series (an infrageneric category) may fall in different pollen types or vice versa. However, pollen morphology was significantly helpful at specific level within each pollen type. A strong correlation was found between the aperture configuration and corolla type but without any correlation between the pollen type and the corolla type.

Key words: *Pedicularis albida*-type, *Pedicularis oederi*-type, *Pedicularis bicornuta*-type, *Pedicularis roylei*-type

Introduction

Pedicularis L. is the largest genus of the family Orobanchaceae, mainly distributed in the mountain ranges of North Temperate Zone of Eurasia, North America and Mexico (Wang *et al.*, 2003; Mill, 2015). There is a controversy about the number of species. According to Fischer (2004) and Mabberley (2008) there are 600 species in the genus, whereas Mill (2015) accepts 750 species. The genus was previously placed in the family Scrophulariaceae. According to recent investigations it is now treated under the family Orobanchaceae in view of its hemiparasitic nature and molecular evidence, particularly on the basis of phytochrome A (Bennett & Mathews, 2006). However, still some of the workers treat *Pedicularis* under the family Scrophulariaceae (Fischer, 2004; Mill, 2015). Hutchinson (1969) and Cronquist (1981) were of the opinion that *Pedicularis* along with the other hemiparasitic members of tribe Rhinanthae of Scrophulariaceae constituted a bridge between Scrophulariaceae and Orobanchaceae. Species of *Pedicularis* are hemiparasitic annuals or perennial herbs and have a great diversity and variation in floral morphology particularly of corolla. This great diversity and strong zygomorphy of the corolla, an adaptation for insect pollination, is not found in any angiospermic flower (Pennell, 1948; Wang *et al.*, 2003). Pollen morphology of several parasitic or hemiparasitic taxa of Orobanchaceae (previously included in tribe Rhinanthae) have been studied by number of workers such as Erdtman (1952) examined the pollen of two species of *Pedicularis* L. and *Lathraea* L. Minkin & Eshbaugh (1989) examined the pollen grains of 57 species of the tribe Rhinanthae of Scrophulariaceae and Orobanchaceae using light and Scanning Electron Microscope. Qaiser *et al.*, (2014) studied the pollen of the genus *Euphrasia* from Pakistan and Kashmir and recognized three pollen types on the basis of exine ornamentation. Pollen morphology of *Pedicularis* species from different parts of the world has been studied from time to time with the objective to provide additional micro morphological characters in this intricate genus which has diverse morphological features and little character differences and also to reveal relationships between the taxa particularly at

infra-generic level. Tsoong & Chang (1965) conducted pollen morphological studies of *Pedicularis* and used the palynological data in the infra-generic classification. Belkina (1972) also investigated the pollen morphology of some Russian species of *Pedicularis*. Belyaeva (1986) studied pollen morphology of 36 species of *Pedicularis* of Siberia and recognized 4 pollen types based on the number and type of colpi and the pollen size. Wang *et al.*, (2003) studied the pollen of 32 Chinese species of *Pedicularis* using light and Scanning Electron Microscope and recognized 3 pollen types, 7 sub-pollen types and correlated these types with the infra-generic classification of the genus. Yu & Wang (2008) investigated palynological characters of *Pedicularis* species belonging to the subgenus *Cyathophora*, endemic to Eastern Himalaya and Hindugan Mountains region. Pollen morphology in relation to floral types and pollination syndrome of 23 species of *Pedicularis* distributed in China, Japan and North America was studied by Wang *et al.*, (2009). Peregrym *et al.*, (2011) studied pollen of 10 Ukrainian species of *Pedicularis* and recognized 3 pollen types on the basis of number of colpi, which were further divided into number of subtypes on the basis of sculpturing type. Mehrvarz *et al.*, (2013) investigated pollen morphology of 9 Iranian species of *Pedicularis* and divided them into two main subtypes on the basis of apertures and exine sculpturing.

In his recent treatment of the family Scrophulariaceae for Flora of Pakistan, Mill (2015) recognized thirty-two species of the genus *Pedicularis* from Pakistan and Kashmir. There are no exclusive reports on the pollen morphology of *Pedicularis* species from the area under consideration with the exception of Bano *et al.*, (2012) who studied the pollen of 4 endemic species of alpine zone of Deosai plateau – Himalayan range. The objective of the present study was (i) to provide a detailed information of pollen morphology of 22 species of *Pedicularis*, out of 22 species, pollen of 16 species are examined for the first time of which 6 species are narrow endemic to the area under consideration (Table 1), (ii) also to find out additional micro-morphological characters in this intricate genus and (iii) finally to establish a correlation between the pollen morphological characters and infra-generic classification.

Table 1. List of *Pedicularis* species, endemic to Pakistan and Kashmir.

Species	Series	Locality
<i>P. elephantoides</i> Benth.	Bicornutae	Hazara and Kashmir
<i>P. karakorumiana</i> T. Yamaz	Sudeticae	Gilgit
<i>P. kashmiriana</i> Pennell	Tenuirostres	Pakistan and Kashmir
<i>P. multiflora</i> Pennell	Tenuirostres	Swat and Kashmir
<i>P. mureeana</i> R.R. Mill & R. Bone	Tenuirostres	Murree-Rawalpindi
<i>P. staintonii</i> R.R. Mill	Tenuirostres	Chitral and Gilgit

Materials and Methods

Polleniferous material was obtained from the Herbarium specimens of Karachi University Herbarium (KUH). In rare cases fresh material was also collected from the field. The list of voucher specimens is deposited in KUH (Karachi University Herbarium, Centre for Plant Conservation). For light microscopy slides were prepared according to classic acetolysis method described by Erdtman (1952). Observations were made under Nikon light microscope 40x and 100x oil immersion. Following measurements were taken: Polar axis (P), Equatorial diameter (E), Aperture size, Exine thickness. For each species 20 readings were taken.

Scanning Electron Microscopy (SEM): Standard procedure was followed (Perveen & Qaiser, 2007, 2008). The pollen were suspended in a drop of water and then transferred on a metallic stubs using double sided adhesive cellotape and coated with gold at 150°A in a

sputtering chamber (ion sputter JFC-1100). Pollen were examined on a Jeol microscope JSM-6820. The terminology used is in accordance with Erdtman (1952), Faegri & Iversen (1964), Kremp (1965), Walker & Doyle (1975) and Punt *et al.*, (2007).

Results

General pollen characters of the genus *Pedicularis*:

Pollen grains are usually radially symmetrical, isopolar, oblate spheroidal, prolate-spheroidal, sub-prolate rarely prolate, tri-syncolpate or bi-syncolpate, outline in polar view is trilobate, bilobate, elliptic or circular. Sexine slightly thicker or thinner than nexine. Tectum varies from psilate to micro scabrate-punctate, medium scabrate punctate, microfoveolate-rugulate.

On the basis of exine ornamentation four distinct pollen types are recognized viz., *P. albida*-type, *P. bicornuta*-type, *P. roylei*-type, and *P. oederi*-type.

Key to the pollen types

1. ± Tectum microfoveolate-rugulate *P. oederi*-type
 - Tectum not as above 2
2. ± Tectum micro-medium scabrate 3
 - Tectum psilate *P. bicornuta*-type
3. ± Tectum microscabrate-punctate *P. albida*-type
 - Tectum medium scabrate-punctate *P. roylei*-type

Pollen type-I: *Pedicularis albida* (Fig. 1, A-F; Fig. 2, A-B; Table 2).

Pollen class: Bi-syncolpate, rarely tri-syncolpate

P/E ratio: 1.03-

Shape: Oblate-spheroidal or prolate-spheroidal rarely sub-prolate.

Apertures: More or less elongated.

Exine: Sexine thinner or thicker than nexine.

Tectum: Micro scabrate punctuate

Measurements: Polar axis P (15.26-) 19.58 ± 1.07 (-23.91) µm, equatorial diameter E (14.46-) 18.98 ± 1.9 (-23.5) µm, Colpus (13.3-) 17.20 ± 1.09 (-21.11) µm in length. Mesocolpium 13.5-19.2 µm. Sycolpate. Exine 1.31-2.60 µm thick, sexine thinner than nexine.

Species included: *P. albida* Pennell, *P. bipinnatifida* (Pennell) R.R. Mill., *P. dolichorhiza* Schrenk, *P. karakorumiana* T. Yamaz., *P. pectinata* Wall ex Benth., *P. pycnantha* Boiss., *P. stewartii* Pennell and *P. staintonii* R.R. Mill.

Key to the species

1. ± Pollen bisyncolpate 2
 - Pollen trisyncolpate 5
2. ± Pollen oblate-spheroidal 3
 - Pollen prolate-spheroidal 4
3. ± Equatorial diameter 21.04-23.67 µm. Mesocolpium 21-23 µm *P. bipinnatifida*
 - Equatorial diameter 13.13-14.0 µm. Mesocolpium ca 14.48 µm *P. dolichorhiza*
4. ± Equatorial diameter 21-25 µm, colpus length ca 21.11 µm, Mesocolpium ca 20.2 µm *P. pectinata*
 - Equatorial diameter 17.3-18.41 µm, colpus length ca 19.72 µm, Mesocolpium ca 19.2 µm *P. staintonii*
5. ± Pollen oblate spheroidal *P. karakorumiana*
 - Pollen subprolate-prolate spheroidal 6
6. ± Pollen subprolate, polar diameter 21.04-22.5 µm, colpus length 18.67-19.70 µm *P. pycnantha*
 - Pollen prolate – prolate spheroidal, colpus length 13.15 – 17.04 µm 7
7. ± Pollen prolate, polar axis 15.78 – 18.6 µm *P. albida*
 - Pollen prolate spheroidal, polar axis 15.26 – 15.78 µm *P. stewartii*

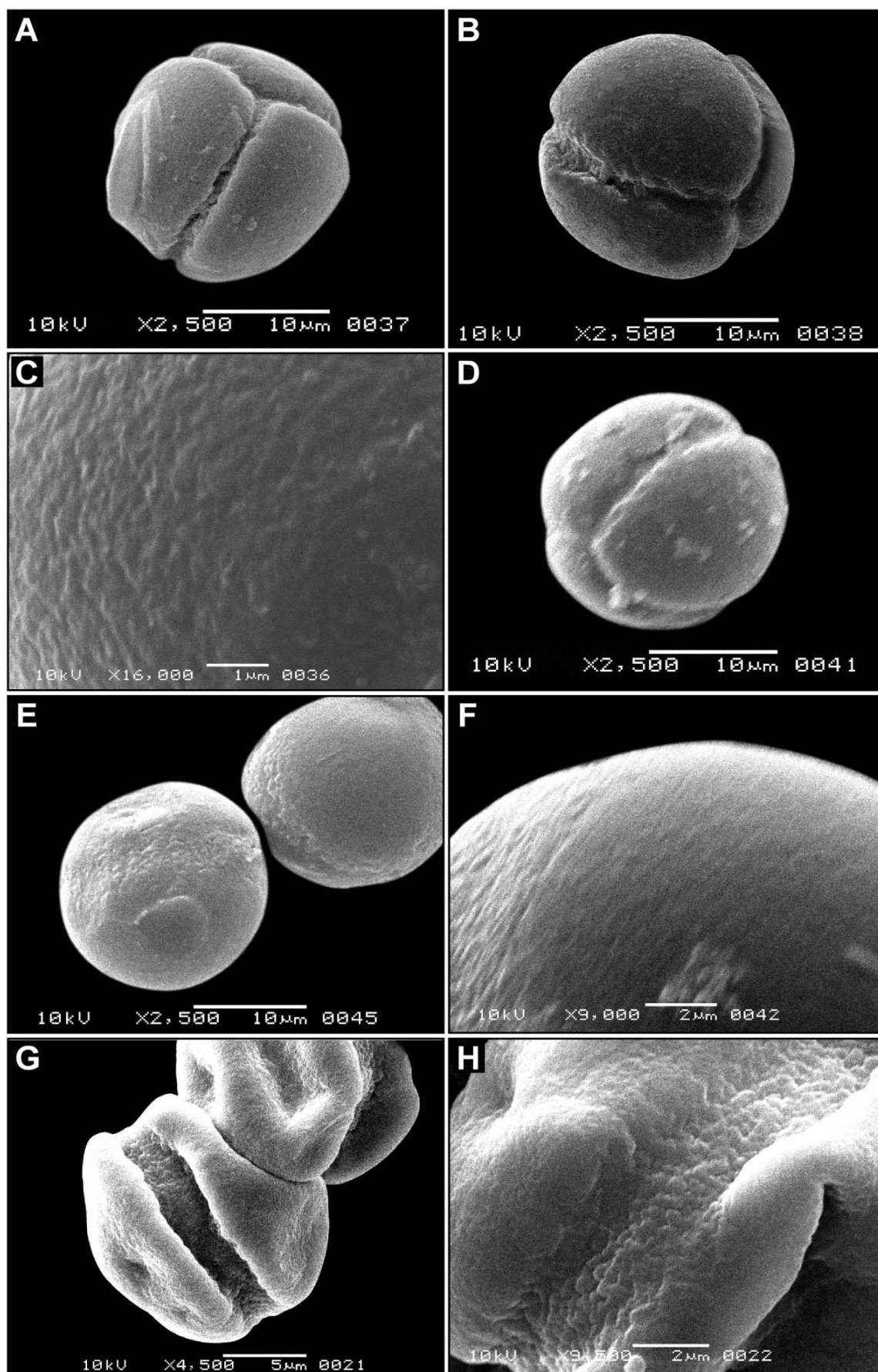


Fig. 1. Scanning electron micrograph: *Pedicularis albida*: A, Polar view, B, Equatorial view, C, Exine pattern, *Pedicularis pycnantha*: D, Polar view, E, Equatorial view; F, Exine pattern. *P. stewartii*: G, Equatorial view, H, Exine pattern. Scale bar: A, B, D, E=10 µm; C = 1 µm; F & H = 2 µm; G = 5 µm

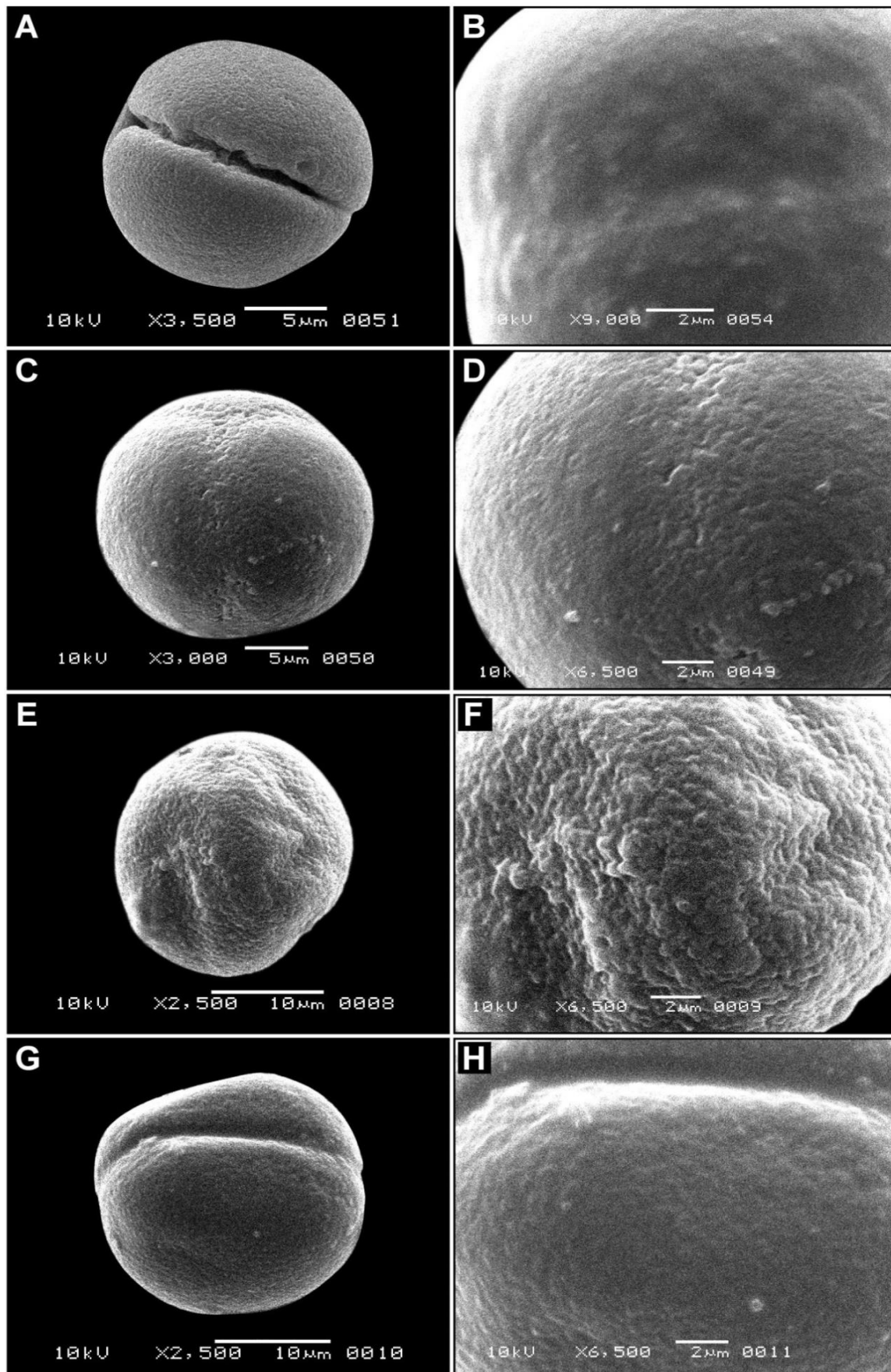


Fig. 2. Scanning electron micrographs: *Pedicularis karakorumiensis*: A, Equatorial view, B, Exine pattern. *Pedicularis oederi*, C, Equatorial view, D, Exine Pattern, *Pedicularis brevifolia*: E, Equatorial view, F, Exine pattern. *P. punctata*: G, Equatorial view, H, Exine pattern.

Scale bar: A & C = 5 μm; B, D, F, & H = 2 μm; E & G = 10 μm

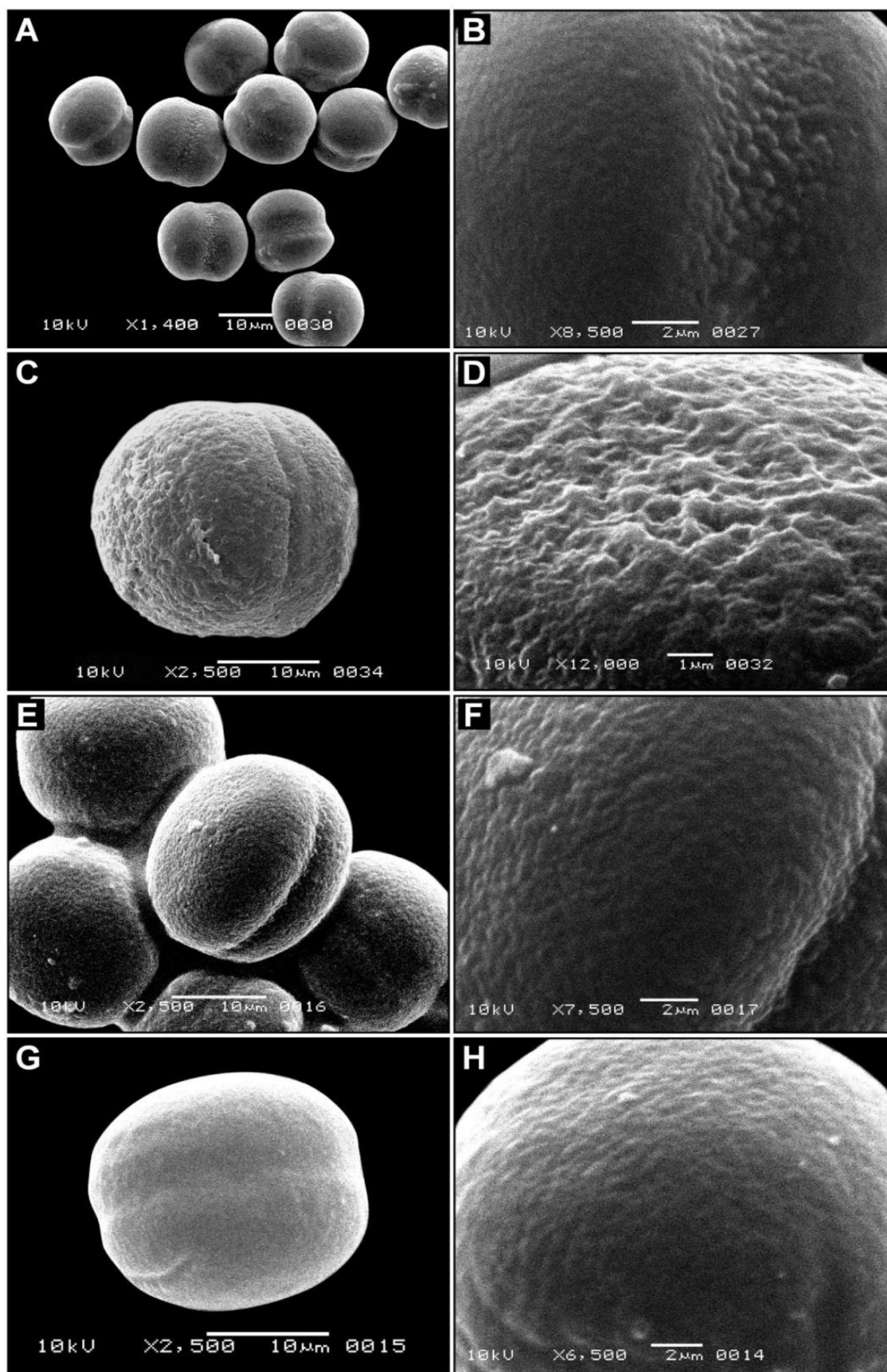


Fig. 3. Scanning electron micrographs: *Pedicularis multiflora*: A, Pollen grains, B, Exine pattern. *Pedicularis elephantoides*: C, Equatorial view, D, Exine pattern, *Pedicularis kashmiriana*: E, Equatorial view, F, Exine pattern. *P. rhinanthoides*: G, Equatorial view, H, Exine pattern.
 Scale bar: A, C, E & G = 10 µm; B, F & H = 2 µm; D = 1 µm

Pollen Type-II: *Pedicularis bicornuta* (Table 3)**Pollen class:** Tri-syncolpate**Shape:** Oblate-spheroidal or prolate-spheroidal**Apertures:** More or less elongated colpus.**Exine:** Sexine thinner or thicker than nexine.**Tectum:** psilate**Measurements:** Polar axis P (25.01-) 28.59 ± 1.07 (-31.93) μm , and equatorial diameter E (25.46-) 26.00 ± 1.5 (-27.5) μm , Colpus (-25.00) 27.15 ± 1.21 (-30.17) μm in length. Mesocolpium (13.1-) 14.44 ± 1.83 (-15.78) μm . Syncolpate. Exine (1.25) 1.62 (-2.11) μm thick, sexine thinner than nexine. Tectum psilate.**Species included:** *P. bicornuta* Kl, *P. svenhedinii* Pennell**Key to the species**

1. \pm Pollen oblate-spheroidal, polar axis 23.1-27.0 μm *P. bicornuta*
 - Pollen prolate-spheroidal, polar axis 27.1-31.0 μm *P. svenhedinii*

Pollen type-III: *Pedicularis oederi* (Fig. 2, C-F & Fig. 3, A-B, Table 4).**Pollen class:** Bi-syncolpate or trisyncolpate**P/E ratio:** 1.13-1.31**Shape:** Prolate - Oblate-spheroidal to prolate-spheroidal rarely sub-prolate**Apertures:** Elongated colpus**Exine:** Sexine thinner or thicker than nexine.**Tectum:** microfoveolate-rugulate**Measurements:** Polar axis P (15.1-) 23.95 ± 0.07 (-32.81) μm , and equatorial diameter E(16.04-) 21.09 ± 1.9 (-26.05) μm . Colpus 15-19.0 μm in length. Mesocolpium 14.00-32.00 μm . Syncolpate. Exine 1.31-1.84 μm thick, sexine thinner than nexine.**Species included:** *P. brevifolia* D.Don., *P. punctata* Dcne., *P. multiflora* Pennell and *P. oederi* Vahl**Key to the species**

1. \pm Pollen oblate-spheroidal *P. oederi*
 - Pollen prolate-spheroidal to subprolate 2
 2. \pm Pollen sub-prolate *P. multiflora*
 - Pollen prolate, prolate-spheroidal 3
 3. \pm Pollen prolate. Polar length 28.93-32.81 μm *P. punctata*
 - Pollen prolate spheroidal. Polar length 23-26 μm *P. brevifolia*

Pollen type-IV: *Pedicularis roylei* (Fig. 3, C-H, Table 5).**Pollen class:** Bi-syncolpate rarely tri-syncolpate**P/E ratio:** 0.89-1.04**Shape:** Oblate-spheroidal to Prolate- spheroidal rarely prolate**Apertures:** Elongated colpus**Exine:** Sexine thinner than nexine.**Tectum:** medium scabrate-punctate**Measurements:** Polar axis P(18.1-) 20.75 ± 5.07 (-30.5) μm and equatorial diameter E(15.78-) 23.8 ± 1.9 (-36.11) μm . Colpus (15.7-) 27.60 ± 1.09 (-23.93) μm in length. Mesocolpium 13-30 μm . Syncolpate. Exine 1.31 μm thick sexine thinner than nexine. Tectum medium scabrate-punctate.**Species included:** *P. elephantoides* Benth., *P. purpurea* Pennell, *P. kashmiriana* Pennell, *P. longiflora* Rudolph, *P. pyramidata* Royle, *P. roylei* Maxim., *P. rhinanthoides* Schrenk ex Fisch. & Mey., and *P. murreeana* R.R. Mill & R. Bone.**Key to the species**

1. \pm Pollen trisyncolpate 2
 - Pollen bisyncolpate 3
 2. \pm Pollen subprolate *P. purpurea*
 - Pollen prolate spheroidal *P. rhinanthoides*
 3. \pm Pollen oblate spheroidal 4
 - Pollen subprolate-prolate spheroidal 6
 4. \pm Polar axis 27-30.5 μm , equatorial diameter 31.11-36.11 μm *P. longiflora*
 - Polar axis 18-23.93 μm , equatorial diameter 20-24.67 μm 5
 5. \pm Mesocolpium ca 13.5 μm *P. elephantoides*
 - Mesocolpium 19-22 μm *P. murreeana*, *P. kashmiriana*
 6. \pm Pollen subprolate, colpus length 15.96-23.67 μm *P. roylei*
 - Pollen prolate-spheroidal, colpus length 15.78-15.88 μm *P. pyramidata*

Table 2. General characters of pollen in *Pedicularis albida* type

Name of species	Polar axis (P) in μm	Equatorial diameter in μm	Shape	Aperture	Colpus length μm	Mesocolpium μm	Apocolpium μm	Exine thickness	Tectum
<i>Pedicularis albida</i> Pennell	16.00(17.35)18.61	15.78(17.38)18.93	Pr	Tri	13.15(15.09)17.04	ca 13.5	Syncolpate	1.31	Micro scabrate punctate
<i>P. bipinnatifida</i> (Pennell)R. R. Mill	18.41	21.04(22.35)23.67	Ob-sp	Bi	15.78	21-23	Syncolpate	2.63	Micro scabrate punctate
<i>P. dolichochariza</i> Schrenk	15.78(-17.22)18.67	17.59(-18.00)18.41	Ob-sp	Bi	13.13 (13.71) -14.00	ca 14.48	Syncolpate	1.31	Micro scabrate punctate
<i>P. pectinata</i> Wall ex Benth.,	18.5 (19.75) 21.00	21.00(22.00)25.00	Pr-sp	Bi	c. 21.11	ca 20.22	Syncolpate	1.30	Micro scabrate punctate
<i>P. karakorumiana</i> T. Yamaz.	18.41(18.54)18.67	18.41(18.51)18.62	Ob-sp	Tri	15.78 (16.83)17.88	ca 17.88	Syncolpate	1.31	Micro scabrate punctate
<i>P. pycnantha</i> Boiss.,	21.04(22.48)23.93	21.04(21.79)22.55	Sub-pr	Tri	18.67(19.06)19.70	15.78	Syncolpate	1.30	Micro scabrate punctate
<i>P. staintonii</i> R.R. Mill	18.41(19.72)21.04	17.3 (17.85)18.41	pr-sp	Bi	19.72	19.2	Syncolpate	1.31	Micro scabrate punctate
<i>P. stewartii</i> Pennell	15.26(15.52)15.78	14.46(15.38)16.30	pr-sp	Tri	13.50(14.64)15.78	13.15	Syncolpate	1.31	Micro scabrate punctate

Table 3. General pollen characters of pollen in *Pedicularis bicornuta* type

Name of species	Polar axis (P) in μm	Equatorial diameter in μm	Shape	Aperture	Colpus length μm	Mesocolpium μm	Apocolpium μm	Exine thickness	Tectum
<i>Pedicularis bicornuta</i> Pennell	23.11(25.01)27.00	26.4(27.25)28.1	Ob-sp	Tri	ca 27.00	ca 13.5	Syncolpate	1.32	Psilate
<i>Pedicularis svenhedinii</i> Pennell	27.11 (29.05)31.00	28.4 30.75) 33.11	Pr-Sp	Tri	ca 21.11	21-23	Syncolpate	1.73	Psilate

Table 4. General characters of pollen in *Pedicularis oederi* type

Name of species	Polar axis (P) in μm	Equatorial diameter in μm	Shape	Aperture	Colpus length μm	Mesocolpium μm	Apocolpium μm	Exine thickness	Tectum
<i>Pedicularis brevifolia</i> D. Don	23.67(24.98)26.03	23.69(24.33)24.97	Pr-sp	Bi	ca 21.00	ca 24	Syncolpate	ca 1.59	Micro-foveolate-rugulate
<i>P. multiflora</i> Pennell	15.78 (-17.22)18.67	14.59 (-15.38)15.41	Ob-sp	Bi	ca 15.78	ca 17.88	Syncolpate	ca 1.51	Micro-foveolate-rugulate
<i>P. oederi</i> Vahl	18.41(18.54)18.67	18.41(18.41)18.62	Pr-sp	Bi	15.78(16.83)17.88	ca 14-18	Syncolpate	ca 1.31	Micro-foveolate-rugulate
<i>P. punctata</i> DC. ex.	28.93(30.87)32.81	19.72(22.88)26.05	pr	Bi	18.67(18.87)19.70	ca 30.24	Syncolpate	ca 1.84	Micro-foveolate-rugulate

Table 5. General pollen characters of pollen in *Pedicularis roylei* type

Name of species	Polar axis (P) in μm	Equatorial diameter in μm	Shape	Aperture	Colpus length μm	Mesocolpium μm	Apocolpium μm	Exine thickness	Tectum
<i>Pedicularis elephantooides</i> Benth.	22.40(23.16)23.93	23.98(24.32)24.67	Ob-sp	Bi	21.04(22.48)23.93	ca 13.5	Syncolpate	1.31	Medium scabrate
<i>Pedicularis longiflora</i> Rudolph	27.00(28.00)30.5	31.11(33.5)36.11	Ob-sp	Bi	C28.11	ca 30.00	Syncolpate	1.31	Medium scabrate
<i>P. kashmiriana</i> Pennell	18.40(19.85)21.30	20.63(21.43)22.24	Ob-sp	Bi	18.20(19.85)21.50	19.22	Syncolpate	1.31	Medium scabrate
<i>P. purpurea</i> Pennell	22.35(-24.32)26.30	18.41(-20.48)22.55	Sub-pr	Tri	18.67 (21.17) -23.67	ca 14.48	Syncolpate	1.31	Medium scabrate
<i>P. pyramidata</i> Royle	18.14(18.27)18.41	15.78(17.09)18.41	Pr-sp	Bi	15.78 (13.54)15.88	ca 17.88	Syncolpate	1.31	Medium scabrate
<i>P. rhinanthoides</i> Schrenk ex Fisch. & Mey.	ca 23.67	ca 21.0	Pr-sp	Tri	18.67(19.06)18.70	15.78	Syncolpate	1.30	Medium scabrate
<i>P. roylei</i> Maxim.	18.41(19.84)21.54	21.64(22.52)23.40	Sub-pr	Bi	15.96(19.81)23.67	19.2	Syncolpate	1.31	Medium scabrate
<i>P. murrexana</i> R. R. Mill & R. Bone	19.30(19.98)20.67	21.63(22.76)23.90	Ob-sp	Bi	18.67(19.88)21.40	19-23	Syncolpate	1.31	Medium scabrate

Abbreviation: Ob-sp = Oblate spheroidal, Sub-pr = Subprolate; Pr-sp= Prolate spheroidal, pr = Prolate

Discussion

Pedicularis, an eurypalynous taxon, exhibited variation in number of pollen characters including the shape, number and type of aperture and the exine ornamentation. However, apertural types and exine were the most significant pollen characters. On the basis of exine ornamentation the genus was divided into four pollen types viz., *Pedicularis albida* - type; *Pedicularis bicornuta*-type, *Pedicularis roylei*-type, and *Pedicularis oederi*-type. Within the genus both bisyncolpate and trisyncolpate pollen were found. However, most of the studied species (63.6%) such as, *Pedicularis bipinnatifida* (Pennell) R.R. Mill., *P. brevifolia* D. Don, *P. punctata* Dcne., *P. multiflora* Pennell, *P. oederi* Vahl, *P. dolichorhiza* Schrenk, *P. elephantoides* Benth., *P. longiflora* Rudolph, *P. pyramidata* Royle, *P. roylei* Maxim., *P. rhinanthoides* Schrenk ex Fisch. & Mey., *P. murreeana* R.R. Mill. & R. Bone and *P. staintonii* R.R. Mill., had bisyncolpate pollen whereas, remaining eight species (36.4%) had trisyncolpate pollen viz., *Pedicularis albida* Pennell, *P. karakorumiiana* T. Yamaz., *Pedicularis bicornata* KI, *P. svenhedinii* Pennell, *P. pycnantha* Boiss. *P. purpurea* Pennell, *P. stewartii* Pennell and *P. rhinanthoides* Schrenk ex Fisch. & Mey. Tsoong & Chang (1965) and Wang *et al.*, (2003) recognized three types of aperture in the Chinese species of *Pedicularis* i.e., tri-colpate, trisyncolpate and bi-syncolpate. Peregrym *et al.*, (2011) also reported 3 types of aperture in Ukrainian species. However, in the present studies only two types of apertures viz., bisyncolpate and trisyncolpate were found. None of the studied taxa had tricolpate pollen. Tsoong & Chang (1965) were of the opinion that the ancestral pollen type in this genus was tri-colpate, which was usually present in some 'primitive' or early diverging species. This was supported by data from gross morphology of the genus, corresponding either to alternate leaves or to the galea with a densely bearded margin (Li, 1951). However, Dutta & Chanda (1979) were of the opposite view and considered bicolpate pollen were primitive than tricolpate pollen in the genus *Pedicularis*, further more they suggested that bicolpate pollen with obscure surface were more primitive with psilate surface. However, the contention of Dutta & Chanda (1979) was not supported by the morphological features. Bicolpate pollen are present in those *Pedicularis* species which have advanced floral characters indicating that the different characters are evolved independently at different rate under different environmental pressure. Varghese (1968) reported bisyncolpate pollen only in the genus *Pedicularis* and absent from the other genera of Scrophulariaceae/ Orobanchaceae. Minkin & Eshbaugh (1989) suggested that the most primitive non-parasitic plants had mostly colporate pollen and in the parasitic plants the circular/endoapertures were secondarily reduced which were either indistinct or even absent. Ree (2005) using molecular data also showed that all the early diverging clades of this genus possessed tri-colpate pollen.

Exine ornamentation is also variable in the genus *Pedicularis*. Within the genus four types of exine ornamentation were found viz., micro-scabrate-punctate, -medium scabrate-punctate, micro foveolate-rugulate and psilate. However, Wang *et al.*, (2003) reported five types of

exine ornamentation: i.e., microfoveolate, microreticulate, microrugulate, microscabrate and retipilate in the genus *Pedicularis*. Peregrym *et al.*, (2011) reported seven subtype of exine pattern within the genus *Pedicularis*, such as, 3-syncolpate pilate, 3-syncolpate microscabrate-tubeculate, 2-syncolpate micro-scabrate, 2-syncolpate microscabrate-tubeculate, 2-syncolpate microscabrate-tubeculate-perforate, 2-syncolpate microfoveolate, and 2-syncolpate microscabrate-perforate, Wang *et al.*, (2003) suggested that the genus *Pedicularis* was heterogeneous in its apertural types and the sculpturing pattern. Out of the 4 types of exine pattern viz., microscabrate-punctate, psilate, microfoveolate-rugulate and medium scabrate-punctate recognized in the present study, of which microfoveolate-rugulate exine is reported for the first time in the genus *Pedicularis*. Bano *et al.*, (2012) also reported microscabrate and psilate exine ornamentation while studying the four endemic species of *Pedicularis* from Alpine zone of Deosai plateau Himalayan region. However, during the recent treatment by Mill (2015) two species studied by Bano *et al.*, (2012) were no more endemic as they were also reported from other areas.

Almost every worker based their infrageneric classification of *Pedicularis* on the variation of corolla and phyllotaxy. Though several infrageneric classification of *Pedicularis* have been proposed by the earlier workers and divided the genus into section/subgenera and series (Mill, 2001). Bunge (1844), Li (1949) and Yamazaki (1988) used the term of subgenera whereas Prain (1980) divided the genus into number of series. Following Prain (1890), Tsoong (1955) preferred the term series over subgenera/section. Mill (2015) in view of wide use of term series also used it in Flora of Pakistan and recognized 15 series. The studied 22 species of the genus *Pedicularis* fall under 11 series Ser. 1. Cheilanthifoliae (*P. albida*, *P. purpurea*, *P. svenhedinii*, Ser. 2. Pycnanthae (*P. pycnantha*, Ser. 3. Verticillatae (*P. roylei*), Ser. 4. Brevifoliae (*P. brevifolia*), Ser. 5. Tenuirostres (*P. bipinnatifida*, *P. kashmiriana*, *P. pectinata*, *P. staintonii*, *P. stewartii*, *P. pyramidata* and *P. multiflora*), Ser. 6. Flammeae (*P. oederi*), Ser.7. Sudeticae (*P. karakorumiiana*), Ser. 8. Comosae (*P. dolichorhiza*), Ser. 9. Rhinanthoides (*P. rhinanthoides*), Ser. 10. Longiflorae (*P. longiflora*, *P. punctuata*) and Ser. 11. Bicornutae (*P. bicornata*, *P. elephantoides*).

The pollen types do not correspond with the infrageneric classification. Members of different series belong to the same pollen type e.g., pollen type *P. albida* have species of five different series viz., Cheilanthifoliae, Comosae, Sudeticae, Pycnanthae and Tenuirostres. Similarly, six species belonging to one pollen type are distributed in six different series. However, to some extent species of the same series, if present within the same pollen type, can be distinguished on the basis of pollen characters. For instance in the series Tenuirostres, *P. kashmiriana* and *P. pyramidata* can be differentiated on the basis of pollen shape. Similar results were also obtained by the cluster analysis. A dendrogram based on 16 pollen characters was constructed using ward's linkage rescaled distance cluster combine (Fig. 4) Two main clades were recognized. Clade-1 was the largest group having 18 species belonging to 9 series viz., Ser. 1. Cheilanthifoliae (*P. albida*, *P. purpurea*)

Ser. 2. Pycnanthae (*P. pycnantha*) Ser. 3. Verticillatae (*P. roylei*), Ser. 4. Tenuirostres (*P. bipinnatifida*, *P. kashmiriana*, *P. pectinata*, *P. staintonii*, *P. stewartii*, *P. pyramidata* and *P. multiflora*), Ser. 5. Flammeae (*P. oederi*), Ser. 6. Sudeticae (*P. karakorumiana*), Ser. 7. Comosae (*P. dolichorhiza*), Ser. 8. Rhinanthoides (*P. rhinanthoides*), Ser. 9. Bicornutae (*P. bicornata*, *P. elephantoides*). This group is usually characterized by beaked and few species with short beaked and beakless corolla. However, this group was further divided into two subgroups A & B. Subgroup A accommodates 12 species. viz., *P. albida*, *P. roylei*, *P. bipinnatifida*, *P. kashmiriana*, *P. murreeana*, *P. staintonii*, *P. stewartii*, *P. pyramidata* and *P. multiflora*), *P. oederi*, *P. karakorumiana* and *P. dolichorhiza*), within the Clad-1 subgroup-A was more or less correlated with the infrageneric classification such as most (seven) of the studied taxa of series Tenuirostres fell in this group whereas, remaining five species were distributed in five series viz., Cheilanthifoliae, Comosae, Flammeae, Sudeticae and Verticillatae each representing single species. Furthermore, with few exception (*P. multiflora* and *P. oederi* had microfoveolate–regulate tectum) majority of the species in this group had micro scabrate punctuate to medium punctuate scabrate tectum.

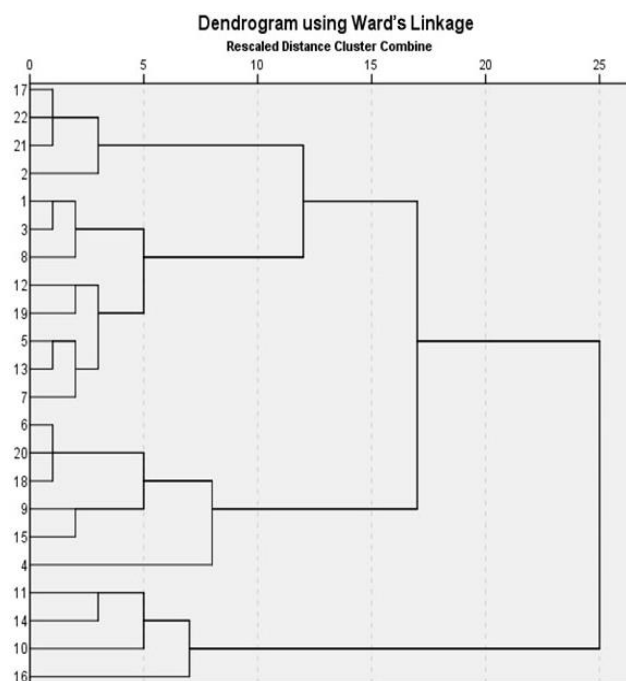


Fig. 4. Dendrogram of 22 *Pedicularis* species based on pollen characters (Ward's linkage, rescaled distance cluster combine)

1. *P. albida*, 2. *P. bipinnatifida*, 3. *P. dolichorhiza*, 4. *P. pecunata*, 5. *P. karakaramiana*, 6. *P. pycnantha*, 7. *P. staintonii*, 8. *P. stewartii*, 9. *P. bicornulata*, 10. *P. svenhedinii*, 11. *P. brevifolia*, 12. *P. multiflora*, 13. *P. oederi*, 14. *P. punctate*, 15. *P. elephantoides*, 16. *P. longiflora*, 17. *P. kashmiriana*, 18. *P. purpureae*, 19. *P. pyramidata*, 20. *P. rhynanthoides*, 21. *P. roylei*, 22. *P. mureccana*

Similarly, group B within Clade-1 also showed correlation with infrageneric classification and five species of this group namely *P. bicornata*, *P. elephantoides*, *P. pectinata*, *P. purpurea* and *P.*

pyramidata, species were distributed in 5 series Bicornuta, Cheilanthifoliae, Rhinanthoides, Pycnanthae and Tenuirostres except series Bicornutae each representing single species. Clade-2 had only four species distributed in 3 series namely Longiflorae (*P. longiflora*, *P. punctuata*), Brevifoliae (*P. brevifolia*) Cheilanthifoliae: *P. svenhedinii*, had beaked and short-beaked corolla.

Pollen aperture configuration is correlated with corolla types. Among 22 species 13 species (*P. bicornuta* Klotzsch, *P. elephantoides* Benth., *P. pyramidata* Royle, *P. rhinanthoides* Schrenk ex Fisch. & Mey. *P. brevifolia* D. Don, *P. punctata* Dcne., *P. multiflora* Pennell, *P. stewartii* Pennell and *P. staintonii* R.R. Mill., *P. bipinnatifida* (Pennell) R.R. Mill., *P. longiflora* Rudolph, *P. kashmiriana* Pennell, *P. pectinata* Wall. ex Benth.), had beaked corolla. Four species (*P. purpurea* Pennell *P. dolichorhiza* Schrenk, *P. karakorumiana* T. Yamaz and *P. svenhedinii* Poulsen) were with short beaked corolla, whereas remaining 5 species had beakless corolla (*P. albida* Pennell, *P. pycnantha* Boiss., *P. oederi* Vahl, *P. roylei* Maxim. and *P. murreeana*). It is interesting to note that with few exceptions (*P. staintonii* R.R. Mill.) 11 species with long beaked corolla had bi-syncolpate pollen, whereas the species with beakless or short beaked corolla had tri-syncolpate pollen. However, two species *P. dolichorhiza* Schrenk and *P. roylei* with short beak and beakless corolla had bi-syncolpate pollen. Unfortunately, simple tricolpate pollen could not be observed in present study. Moreover, the four pollen types recognized in the present study do not correspond with corolla types. In *P. oederi*-type, species having different corollas were found e.g. *P. brevifolia* D. Don, *P. punctata* Dcne., *P. multiflora* Pennell, *P. oederi* Vahl. Similar results were also reported by Wang *et al.*, (2009) while, assessing the relationship between pollen aperture configuration and the corolla types, but demonstrated a strong correlation between the aperture number and type with the length of corolla. However, molecular phylogenetic data of floral characters of *Pedicularis* showed that morphologically similar corollas in different species of this genus were apparently derived independently multiple times, perhaps as a result of adaptive radiation (Ree, 2005), he also observed that the species with similar corollas were visited by similar pollinators - bumblebees having similar foraging patterns whereas corollas having more complex structure might be visited by specialized pollinators. This combination of corolla types, pollen and pollination mechanisms (specialized pollinators) is likely to promote reproductive isolation among sympatric species, and the differentiation of floral characteristics may accelerate speciation in *Pedicularis*.

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