

POLLEN MORPHOLOGY OF FOUR ENDEMIC SPECIES OF *PEDICULARIS* L. FROM ALPINE ZONE OF THE DEOSAI PLATEAU, HIMALAYAN RANGE

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Abstract

The pollen morphology with special reference to exine sculpturing of four species of the genus *Pedicularis* L. has been examined by light and scanning electron microscope. Comparative pollen analysis was made based on the type of pollen, shape in polar and equatorial views, P/E ratio, exine thickness and sculpturing of pollen. In this study, two types of pollen aperture configuration known in the genus were observed i.e., tri-syncolpate and bi-syncolpate. Pollen grains with microscabrate ornamentation were found in bi-syncolpate pollen for the first time. Pollen fertility estimation ranged from 87-95%, which shows that pollen flora of selected species is well established in Alpine zone.

Introduction

The Deosai Plateau (30°00' N, 75°30' E) is located in the north of the main Himalayan range in Baltistan, Pakistan. The altitude of the Deosai Plateau is 4,115 meters above mean sea level. It is among the highest plateau of the world and is above the tree line. It is about 30 km from Skardu and covers an area of almost 5,000 sq km, surrounded by Himalayas and lies close to the magnificent Karakorum mountain range, which include the second highest peak of the world K2 (8,611 m) (Anonymous, 1993). The area is surrounded by snowy mountains exceeding 5,000 meters above mean sea level and suspended glaciers. At an average altitude of 3,500 meter, the Deosai Plateau is declared National Park and protected area for wildlife in 1993 (Anonymous, 1993). Over half of the year (between September and May), Deosai remains snow covered and inaccessible (snow is 7-8 meters deep). There are a number of small lakes and the river valleys sloping southeast. About 342 species of plants belonging to 36 families and 142 genera have been recorded from Deosai. This high level biodiversity on the plateau is due to several reasons including topography, location of the plateau (Junction of 4 major mountain ranges) and local adaptation of its plant and animal species (Woods *et al.*, 1997). Sultana *et al.* (2007) worked on altitudinal distribution of grasses, sedges and rushes of the Deosai Plateau and found that majority of the species were found at high altitude due to availability of plenty of moisture and favourable weather conditions.

Pollen morphological characters have been used for the identification of taxa (Erdtman, 1966). Pehlivan *et al.* (2009) worked on pollen morphology of ten taxa of family Umbelliferae and concluded that the most variable pollen characteristics among the investigated taxa are the polar axis, equatorial axis and colpus length. Köksal *et al.* (2010) studied the pollen grains of *Potentilla recta* L. groups A, B & C and compared them using light microscope (LM), scanning electron microscope (SEM) and transmission electron microscope (TEM) to elucidate their taxonomic position at species or subspecies level.

The genus *Pedicularis* L. belonging to the family Scrophulariaceae comprises of 800 species worldwide (Mill, 2001) and is one of the largest angiosperm genus in the Northern Hemisphere (Mabberley, 1987; Yang *et al.*, 1998). Based on light microscopy (LM), some scattered pollen

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data were reported from European (Risch, 1939; Belkina, 1972) and Indian (Dutta and Chanda, 1979) members of *Pedicularis*. Tsoong and Chang (1965) recognized three types of pollen aperture in the genus, i.e., tri-colpate, trisyncolpate and bi-syncolpate. Details on exine ornamentation start to emerge when pollen from two Turkish (Inceoğlu, 1981) and one Canadian species (Minkin and Eshbaugh, 1989) was reported and illustrated using LM and scanning electron microscopy (SEM). There are no reports on palynological studies of this genus from the Himalayans Range of Pakistan. Four endemic species of *Pedicularis*, namely *P. bicornata* Kl., *P. chielanthifolia* Schrenk, *P. pectinata* Wall. ex Benth. and *P. punctata* Decne are for the first time reported here with reference to palynological diversity by light microscopy (LM) and scanning electron microscopy (SEM).

Materials and Methods

Four *Pedicularis* species used in this study were *P. bicornata* Kl., *P. chielanthifolia* Schrenk, *P. pectinata* Wall. ex Benth. and *P. punctata* Decne. For light microscopy, pollen grains were treated by acetolysis (Erdtman, 1960) and mounted in glycerin jelly. Different parameters were studied including pollen shape, aperture type, exine sculpturing, polar and equatorial diameter, P/E ratio and exine thickness. The pollen fertility estimation was determined according to the technique used by Khan and Stace (1999). The percentage of full stained grains was calculated after staining with a mixture of equal amounts of 1% aceto-carmin and neutral glycerin. The pollen grains were prepared for scanning electron microscopy (SEM) by the methods described by Erdtman (1952). The pollen grains suspended in a drop of water were directly transferred to a double sided tape affixed stub with a fine pipette and coated with gold in a sputtering chamber (Ionsputter JFC-1100). Coating was restricted to 150A. The specimens were examined with Jeol microscope JSM-T200 at 15 kV and photographed.

Results and Discussion

Pollen of all selected four species of *Pedicularis* are remarkably uniform in their pollen characters. Maximum pollen size i.e. 40 µm was found in *P. punctata* in polar view and minimum pollen size 20 µm in polar view was observed in *P. pectinata*. Whereas maximum pollen size in equatorial view was 35 µm in *P. punctata* and minimum was 22.5 µm in *P. pectinata*. Pollen shape in equatorial view also varies including oblate-spheroidal in *P. bicornata* and *P. pectinata* (P/E: 0.90 and 0.88), prolate-spheroidal in *P. chielanthifolia* (P/E:1) and subprolate in *P. punctata* (P/E:1.14) (Table 1).

Table 1. Pollen morphological characters of four *Pedicularis* species.

Taxon	Shape	Aperture type	Polar Diameter (µm)	Equatorial Diameter (µm)	P/E ratio	Exine thickness (µm)	Exine sculpturing	% Fertility
<i>P. bicornata</i> Kl.	OS	tri-syncolpate	25 (24.5-26)	27.5 (26.5-28)	0.90	1.25 (1-1.75)	Psilate	94
<i>P. chielanthifolia</i> Schrenk	PS	tri-syncolpate	30 (28.5-31)	30 (28.5-31)	1	1.87 (1.5-2)	Psilate	87
<i>P. pectinata</i> Wall. ex Benth.	OS	bi-syncolpate	20 (19.5-21)	22.5 (21-23)	0.88	3.12 (2.75-3.75)	Microscabrate	95
<i>P. punctata</i> Decne	SP	bi-syncolpate	40 (39.5-41)	35 (34.5-36)	1.14	2.5 (1.75-2.75)	Microscabrate	90

OS = oblate-spheroidal, PS= Prolate-spheroidal, SP= subprolate

Based on the current study, two types of pollen aperture configuration (Tsoong and Chang, 1965) known in the genus were observed, viz., tri-syncolpate and bi-syncolpate. In the present study, *P. bicornuta* and *P. chielanthifolia* have tri-syncolpate pollen (Fig. 1, 1A & 2A), whereas *P. pectinata* and *P. punctata* have bi-syncolpate pollen (Fig. 1, 3A & 4A). The ancestral pollen type in this genus was considered to be tri-colpate (Tsoong and Chang, 1965; Wang *et al.*, 2003),

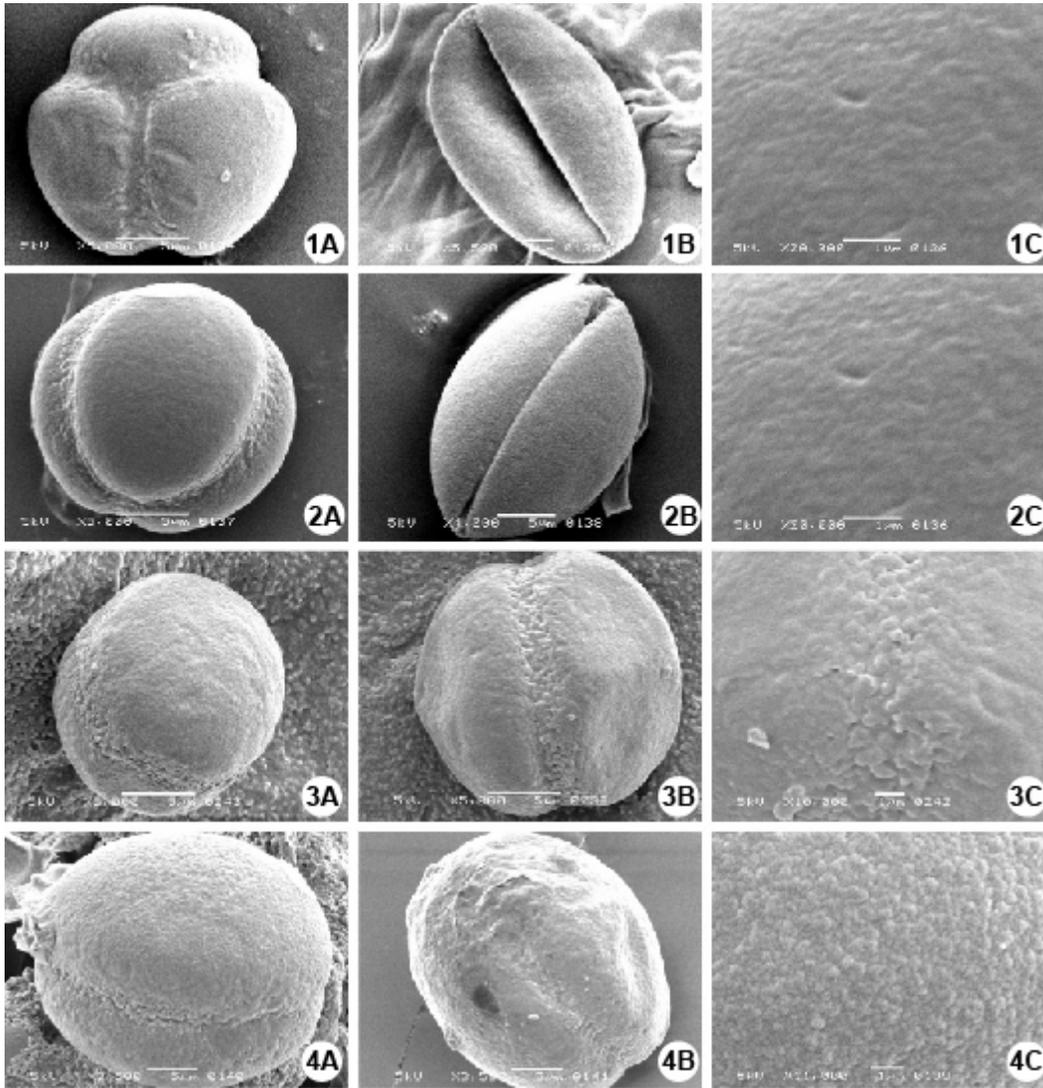


Fig.1. Scanning electron microscope (SEM) micrographs of pollen grains of *Pedicularis* species. 1. *P. bicornuta* (tri-syncolpate) A Polar view, B Equatorial view, C Exine sculpturing. 2. *P. chielanthifolia* (tri-syncolpate) A Polar view, B Equatorial view, C Exine sculpturing. 3. *P. pectinata* A Mesocolpium view, B Equatorial view, C Exine sculpturing. 4. *P. punctata* A Mesocolpium view, B Equatorial view, C Exine sculpturing. (Scales: A+B = 5 μ m, C = 2 μ m).

which is usually present in some 'primitive' or early diverging species. Recent molecular data also revealed that the early diverging clades of this genus possess tri-colpate pollen (Ree, 2005). Moreover, tri-colpate pollen grains do not occur in a monophyletic group *Cyathophora*, which is endemic to the eastern Himalaya-Hengduan Mountains region (Yu and Wang, 2008).

The exine of pollen grains of *Pedicularis* is extremely thin, and it is difficult to distinguish the exine ornamentation under LM (Erdtman, 1960; Beug, 1961; Tsoong and Chang, 1965; Dutta and Chanda, 1979). However, some studies considered that pollen grains in *Pedicularis* are smooth-surfaced (Tsoong and Chang, 1965; Yang *et al.*, 2002), but others found a distinct variation of exine ornamentation (Inceöglu, 1981; Minkin and Eshbaugh, 1989; Wang *et al.*, 2003). The extensive investigation of the exine of some Chinese species of *Pedicularis* using SEM recognised the presence of five types of exine ornamentation: i.e., microfoveolate, microreticulate, microrugulate, microscabrate and retipilate (Wang *et al.*, 2003). SEM observations also revealed three types of aperture configurations, each of which could be subdivided, making a total of eight sub-types (Wang *et al.*, 2003; Yu and Wang 2008). In the present study, the exine ornamentation was psilate with completely smooth surface in *P. bicornuta* and *P. chielanthifolia* (tri-syncolpate) (Fig 1, 1C & 2C). Pollen grains with microscabrate ornamentation were found in bi-syncolpate pollen (*P. pectinata* and *P. punctata*) for the first time (Fig. 1, 3C & 4C). This exine ornamentation was earlier found in species with tri-syncolpate pollen grains according to Wang *et al.* (2009). So far, only 59 species of *Pedicularis* were studied using SEM, therefore, it is difficult to provide relatively complete data of the exine ornamentation in *Pedicularis* (Wang *et al.*, 2009). More studies are still needed in future, utilizing cosmopolitan material, to achieve more conclusive results.

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