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## A palynological study of the genus *Pedicularis* (*Orobanchaceae*) in Iran

### Abstract

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Pollen morphological features of nine Iranian species of the genus *Pedicularis* were examined using light microscopy (LM) and scanning electron microscopy (SEM). We studied the pollen morphology of *P. cabulica*, *P. caucasica*, *P. condensata*, *P. pycnantha*, *P. rechingeri*, *P. rhinanthoides*, *P. sibthorpii*, *P. straussii* and *P. wilhelmsiana*, among which all except *P. condensata*, *P. rhinanthoides* and *P. sibthorpii* are palynologically described here for the first time. We observed two main types of pollen aperture (trisyncolpate and bisyncolpate) and four of exine sculpturing (microscabrate-reticulate, microfoveolate-microscabrate, retipilate and microscabrate). The results reveal the taxonomic significance of palynological characters in the genus, and the taxonomic implications are discussed here.

Additional key words: pollen, SEM, taxonomy

### Introduction

*Pedicularis* L. (*Orobanchaceae*), with 600–800 species, is a large hemiparasitic genus, distributed mostly over boreal and arctic-alpine regions of the northern hemisphere (Mill 2001; Wang & al. 2003; Zhang & Wang 2011). The Sino-Himalayan region harbours more than 50% of the *Pedicularis* species and is an important centre of diversity of the genus (Ree 2001; Wang & Li 2005; Zhang & al. 2006; Yu & Wang 2008; Yu & al. 2008).

There are several taxonomic treatments of the genus dividing it to numerous sections and/or series. The most common treatments consider below the generic level only series (Yang & al. 1998). Due to limited phylogenetic studies available on *Pedicularis* (Ree 2005) and the various methods suggested for the classification of the genus, we do not deal with the infrageneric systems available on *Pedicularis*.

*Pedicularis* is represented by nine species in Iran (Wendelbo 1981), all distributed in the NW, N, NE and C

parts (Saeidi 2006), and among which *P. rechingeri* and *P. straussii* are endemic. *Pedicularis sibthorpii* is widely distributed in Iran, as well as in the Caucasus, Europe, C Asia and Turkey. The species of the genus are herbaceous perennial plants, with pinnatisect and pinnatiparite leaves and flowers arranged in lateral cymes forming a terminal capitulum, raceme or spiciform to verticillate inflorescence. The corolla is 2-lipped; the upper lip or galea is hooded and laterally compressed, rounded or truncate at the apex, or ending in teeth or in a beak; the lower lip is generally 3-lobed.

The utility and importance of fruit features in the determination of the nine Iranian species of this genus was reported by Saeidi & al. (2006). The palynology of *Pedicularis* has already been investigated for a number of species from throughout its distributional range. The most important findings of these previous palynological studies were: (1) three aperture types (tricolpate, bisyncolpate and tri-syncolpate) and (2) various sculpturing types including microscabrate, microrugulate, microre-

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Table 1. Collection data of *Pedicularis* species examined for this study. All refer to herbarium specimens deposited at TARI.

<i>Pedicularis</i> species	Collection data
<i>P. cabulica</i> Benth.	Kerman: Kuhe Lalezar, 3000 m, 17 Jun 1974, <i>Foroughi &amp; Assadi</i> 16331
<i>P. caucasica</i> M. Bieb.	Tehran: Kandevar, 2600–3050 m, 23 Jun 1979, <i>Assadi &amp; Mozaffarian</i> 32881
<i>P. condensata</i> M. Bieb.	Tehran: Kandevar-Haraz road, 2400 m, 24 Jun 1979, <i>Assadi &amp; Mozaffarian</i> 32952
<i>P. pycnantha</i> Boiss.	Tehran: Karaj-Chalus road, 2700 m, 2 Jun 1991, <i>Assadi &amp; Shahsavari</i> 69695
<i>P. rechingeri</i> Wendelbo	Khorasan: Mashhad, Darehgaz, 1720 m, 28 May 1972, <i>Foroughi</i> 5535
<i>P. rhinanthoides</i> Schrenk	Tehran: Gajereh, 500 m, 22 Jul 1984, <i>Aghabeigi</i> 24833
<i>P. sibthorpii</i> Boiss.	Guilan: Roudbar, 2578 m, 28 May 2006, <i>Moradi &amp; Ladani</i> 1180
<i>P. straussii</i> Hausskn. ex Bornm.	Isfahan: Semrom, 1900 m, 24 Jun 2002, <i>Parishan</i> 14500
<i>P. wilhelmsiana</i> Fisch. ex M. Bieb	East Azerbaijan: Arasbaran protected area, 1800 m, 29 May 1977, <i>Assadi &amp; Vosoghi</i> 24982

ticulate, microfoveolate and retipilate (Tsoong & Chang 1965; Dutta & Chanda 1978; Inceoglu 1982; Yang & al. 2002; Wang & al. 2003; Yu & Wang 2008; Peregrym & al. 2011).

Out of the nine species of *Pedicularis* currently known to occur in Iran, data on pollen grains exist for three species only, i.e. *P. condensata*, *P. rhinanthoides*, and *P. sibthorpii*. The aim of the present study was to examine the pollen grains of the Iranian taxa in detail in order to provide palynological information and more data for future taxonomic work.

## Materials and methods

The pollen grains of nine species of *Pedicularis* were studied by light microscopy (LM) and scanning electron microscopy (SEM). The majority of pollen samples were obtained from the Herbarium of the Research Institute of Forests and Rangelands, Tehran (TARI). A list of voucher specimens is given in Table 1. For LM investigation, pollen grains were transferred directly from anthers to glass slides and mounted in glycerine jelly for further observation. Prepared slides were studied with an Olympus BH-2 microscope. Size measurements were based on 25 pollen grains per species. The value of P (polar axis length), E (equatorial diameter), exine thickness and mesocolpium diameter were measured under a  $\times 40$  eyepiece, and the P/E ratio was calculated (Table 2). For SEM investigation, pollen grains were treated with 96 % ethanol and pipetted to double-side tape affixed on 12.5 mm diameter aluminium stubs, and sputter-coated (Emitech k450) with gold. The micrographs were made using an SEM model VEGA/TESCAN at an accelerating voltage of 15 KV under 5000 $\times$  to 30 000 $\times$  magnifications at Razi Metallurgical Research Center (RMRC) in Tehran. The pollen terminology in general follows Erdtman (1952), Punt & al. (2007) and Wang & al. (2003).

## Results

### Description of general pollen morphology

The pollen grains were isopolar, radially symmetrical monads, in shape oblate-spheroidal (P/E = 0.97–1.00), spheroidal (P/E = 0.97–1.14), prolate-spheroidal (P/E = 1.00–1.14) or subprolate (P/E = 1.14–1.16). Their outline varied from subtrilobate to trilobate and subbilobate to bilobate in polar view, and from elliptical to circular in equatorial view. The colpus membrane surface was scabrate or scabrate-tuberculate. Pollen apertures were either trisyncolpate or bisyncolpate. The pollen grain size varied from small to medium (according to Erdtman 1952). The polar axis ranged from  $16.23 \pm 0.98 \mu\text{m}$  (*Pedicularis wilhelmsiana*) to  $34.42 \pm 1.77 \mu\text{m}$  (*P. rhinanthoides*). The equatorial axis ranged from  $14.17 \pm 1.2 \mu\text{m}$  (*P. wilhelmsiana*) to  $29.53 \pm 1.83 \mu\text{m}$  (*P. rhinanthoides*). The P/E ratio ranged from 0.97 (*P. pycnantha*) to 1.16 (*P. rhinanthoides*). The mesocolpium ranged from  $14.67 \pm 1.23 \mu\text{m}$  (*P. pycnantha*) to  $17.63 \pm 2.35 \mu\text{m}$  (*P. caucasica*). Exine was extremely thin and varied from  $0.85 \pm 0.28 \mu\text{m}$  (*P. wilhelmsiana*) to  $1.57 \pm 0.38 \mu\text{m}$  (*P. cabulica*). Pollen apertures and exine sculpturing are two of the most informative pollen morphological features of the genus. The detailed information of pollen morphology for the investigated species is shown in Table 2.

### Pollen type classes

Based on pollen apertures and exine sculpturing, the examined species are divided into two main types and five subtypes.

**Type I:** Trisyncolpate pollen grains (Fig. 1A–I). Two subtypes were identified within this type:

**Subtype Ia:** Microscabrate-reticulate exine sculpturing, observed in *Pedicularis caucasica* (Fig. 1B) and visible only at high magnifications in SEM.

Table 2. Characteristic features of the investigated pollen in Iranian species of *Pedicularis*.

Species	P [µm]	E [µm]	P/E	ET [µm]	ML [µm]	Shape	Aperture	Exine sculpturing	Pollen type and subtypes
<i>P. caucasica</i>	20–25 (23.46 ± 1.58)	22.5–25 (23.75 ± 1.27)	0.98	0.5–1.25 (0.86 ± 0.32)	15–22.5 (17.63 ± 2.35)	OS-S	3-syncolpate	microscabrate- reticulate	Ia
<i>P. pycnantha</i>	17.5–20 (19.01 ± 0.93)	17.5–22.5 (19.53 ± 1.43)	0.97	0.75–1.5 (1.14 ± 0.16)	12.5–16.25 (14.67 ± 1.23)	OS-S	3-syncolpate	retipilate	Ib
<i>P. rechingeri</i>	22.5–25 (23.14 ± 1.08)	22.5–25 (23.1 ± 1.08)	1.00	0.75–2 (1.2 ± 0.32)	15–20 (16.62 ± 1.64)	OS-S-PS	3-syncolpate	retipilate	Ib
<i>P. straussii</i>	20–30 (23.63 ± 1.8)	20–25 (23.73 ± 1.39)	0.99	0.75–2 (1.22 ± 0.29)	12.5–17.5 (16.48 ± 1.36)	OS-S	3-syncolpate	retipilate	Ib
<i>P. condensata</i>	15–17 (16.45 ± 0.97)	12.5–17.5 (15.76 ± 1.34)	1.04	1–2 (1.13 ± 0.27)	–	PS-S	2-syncolpate	microfoveolate- microscabate	IIa
<i>P. wilhelmsiana</i>	15–17 (16.23 ± 0.96)	12.5–15 (14.16 ± 1.2)	1.14	0.5–1.25 (0.85 ± 0.28)	–	PS-S-SP	2-syncolpate	retipilate	IIb
<i>P. sibthorpii</i>	15–20 (17.94 ± 1.13)	16.25–21.25 (18.14 ± 1.21)	0.98	0.75–1.25 (1.05 ± 0.21)	–	OS-S	2-syncolpate	retipilate	IIb
<i>P. cabulica</i>	20–27.5 (24.56 ± 1.74)	20–22.5 (21.79 ± 1.12)	1.12	1–2 (1.57 ± 0.38)	–	PS-S	2-syncolpate	retipilate	IIb
<i>P. rhinanthoides</i>	33–37 (34.42 ± 1.77)	25–32.5 (29.53 ± 1.83)	1.16	1–2.25 (1.52 ± 0.45)	–	SP	2-syncolpate	microscabate	IIc

Abbreviations: P = polar axis length; E = equatorial axis length; P/E = polar/equatorial axis length ratio; ET = exine thickness; ML = mesocolpium length; OS = oblate-spheroidal; PS = prolate-spheroidal; S = spheroidal; SP = subprolate.

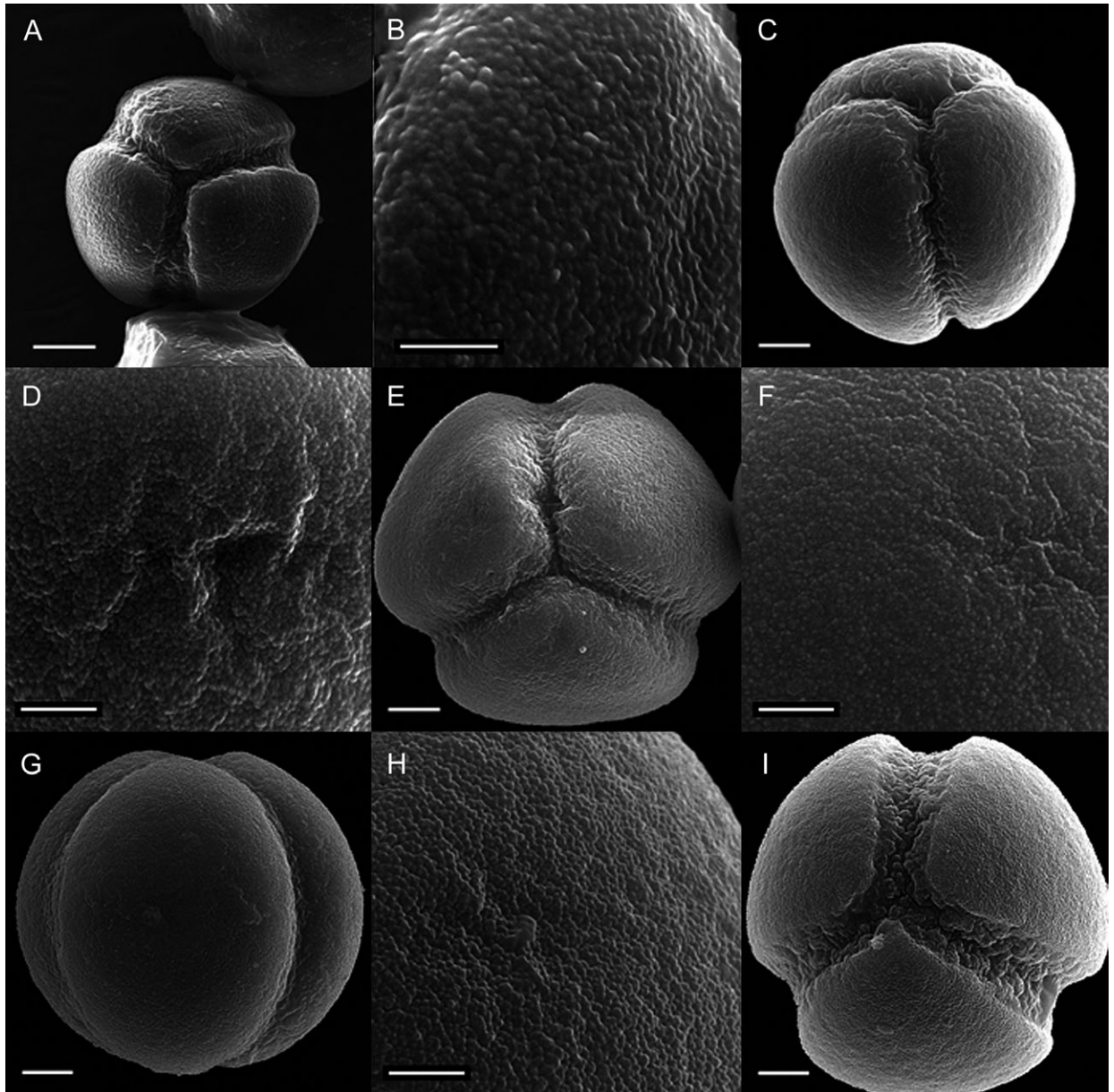


Fig. 1. SEM micrographs of pollen grains in *Pedicularis* species. – A, B: *P. caucasica*; C, D: *P. pycnantha*; E–G: *P. rechingeri*; H, I: *P. straussii*. – A, E, I: polar view; C, G: equatorial view; B, D, F, H: exine sculpturing. – Scale bars: A = 5  $\mu$ m; B, D, F, H = 1  $\mu$ m; C, E, G, I = 2  $\mu$ m.

**Subtype Ib:** Retipilate exine sculpturing, observed in *Pedicularis pycnantha* (Fig. 1D), *P. rechingeri* (Fig. 1F) and *P. straussii* (Fig. 1H).

**Type II:** Bisyncolpate pollen grains (Fig. 2A–I; 3A–F). Three subtypes were identified within this type:

**Subtype IIa:** Microfoveolate-microscabrate exine sculpturing, observed in *Pedicularis condensata* (Fig. 2B). Numerous scabrae situated on the tectum were found at high magnification in SEM.

**Subtype IIb:** Retipilate exine sculpturing, observed in *Pedicularis wilhelmsiana* (Fig. 2E), *P. sibthorpii* (Fig. 2H) and *P. cabulica* (Fig. 3B).

**Subtype IIc:** Microscabrate exine sculpturing, observed in *Pedicularis rhinanthoides* (Fig. 3E).

## Discussion

Palynological analysis of Iranian species of *Pedicularis* identified especially pollen shape, size, aperture types, and exine sculpturing as the most taxonomically informative within the genus. Although *Pedicularis* presents variation in pollen gross morphology, which is in fact one of the features used in the infrageneric classification of the genus, the pollen surface is invariable and relatively smooth in all species (Tsoong & Chang 1965; Dutta & Chanda 1978). *Pedicularis caucasica*, *P. pycnantha*, *P. rechingeri*, and *P. straussii* have trisyncolpate pollen grains, whereas the other studied species have bisyncolpate ones. Tsoong & Chang (1965) as well as Wang & al. (2003) mentioned

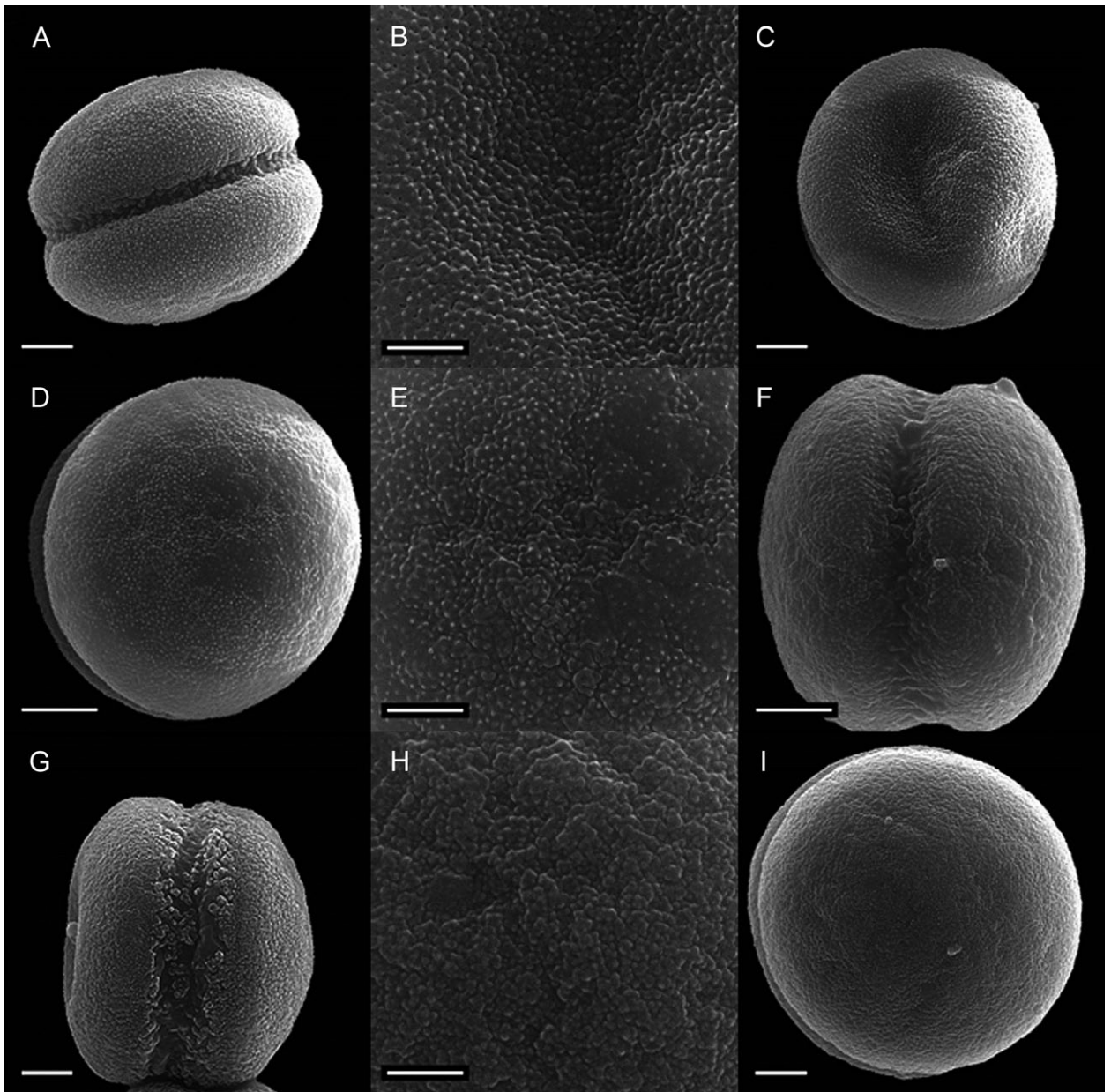


Fig. 2. SEM micrographs of pollen grains in *Pedicularis* species. – A–C: *P. condensata*; D–F: *P. wilhelmsiana*; G–I: *P. sibthorpii*. – A, F, G: equatorial view; C, D, I: mesocolpium view; B, E, H: exine sculpturing. – Scale bars: A, C, D, F, G, I = 2  $\mu$ m; B, E, H = 1  $\mu$ m.

that in the monophyletic *P.* sect. *Cyatophora*, both aperture types can be observed. Therefore, the application of this feature in characterizing closely related species is restricted. However, both species showing the trisyncolpate pollen grains, *P. rechingeri* and *P. straussii*, are characterized also by a verticillate synflorescence and an oblong, symmetrical capsule (Saeidi & al. 2006). Furthermore, the sculpturing on the tectum surface in both species is similar concerning the retipilate sculpturing (Fig. 1F, H).

*Pedicularis pycnantha* resembles *P. caucasica* in having opposite to whorled stem leaves and a falciform-curved corolla tube, but differs from it in calyx length and in the galea spreading backward vs. slightly curved

(not straight) in *P. caucasica* (Saeidi 2006). On the other hand, the exine sculpturing of pollen grains in *P. pycnantha* is retipilate, whereas it is microscabrate-reticulate in *P. caucasica*. Furthermore, the polar and equatorial axis of *P. caucasica* is more than that of *P. pycnantha* (Table 2). Thus, the pollen morphology does not support a close relationship between these species.

The shape of pollen grains varies from oblate-spheroidal, spheroidal, prolate-spheroidal, to subprolate. The subprolate shape was observed only in *Pedicularis cabulica*. With regard to morphological characters, for example the presence of alternate leaves, four taxa resemble each other: *P. cabulica*, *P. rhinanthoides*, *P. sibthorpii*

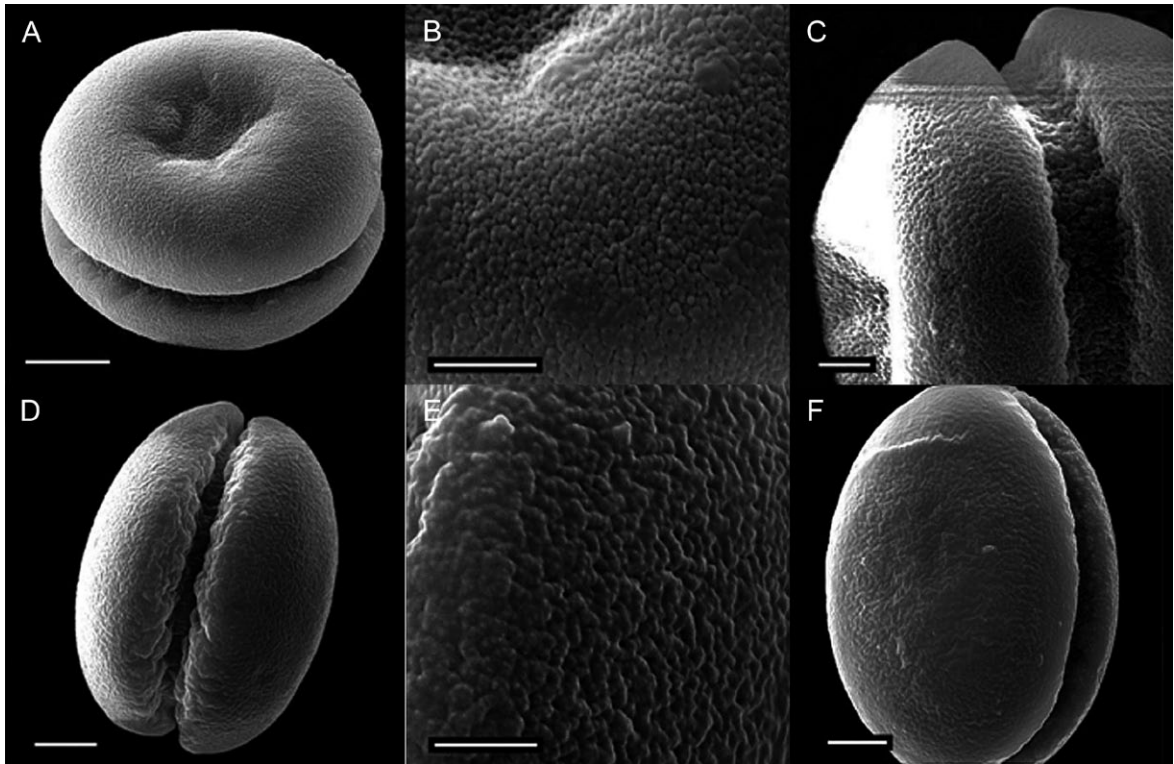


Fig. 3. SEM micrographs of pollen grains in *Pedicularis* species. – A–C: *P. cabulica*; D–F: *P. rhinanthoides*. – C, D: equatorial view; A, F: mesocolpium view; B, E: exine sculpturing. – Scale bars: A, D, F = 5  $\mu\text{m}$ ; B, C, E = 2  $\mu\text{m}$ .

and *P. wilhelmsiana*. *Pedicularis cabulica* can be distinguished on the basis of its corolla colour, purple, vs. yellow, creamy, white or pink in the other three species (Saeidi 2006). The size of the pollen grains can be helpful to distinguish some species, especially *P. rhinanthoides* with the largest pollen grains and *P. wilhelmsiana* with the smallest. Previously, microfoveolate exine sculpturing was found in species with bisyncolpate pollen grains (Wang & al. 2003) and trisyncolpate pollen grains, e.g. *P.* sect. *Cyathophora* (Yu & Wang 2008).

According to our findings, *Pedicularis rhinanthoides* has microscabrate exine sculpturing, which is not in agreement with the previous research of Yang & al. (2002). However, this is not surprising, as the detailed surface features were not studied in that work. In other cases, our results on species such as *P. condensata* and *P. sibthorpii* correspond completely to previous reports (Inceoğlu 1982; Yang & al. 2002; Peregrym & al. 2010).

Pollen morphological features, especially exine sculpturing, is an important feature for delimitation of some species of this genus, which share common morphological features (Saeidi 2006) as well as fruit anatomical traits (Saeidi & al. 2006). According to our results, *Pedicularis condensata* (subtype IIa), *P. rechingeri* (subtype Ib), *P. sibthorpii* (subtype IIb) and *P. straussii* (subtype Ib) have exine sculpturing microfoveolate-microscabrate, retipilate, retipilate and retipilate, respectively (Table 1). Thus, pollen morphology is very useful in species delimitation of *Pedicularis*.

This very large genus is in urgent need of a molecular phylogenetic study (in preparation by Ree, pers. comm.), including sufficient representatives to test the monophyly of the recognized infrageneric taxa. The significance of pollen morphological characters should be discussed in the light of such a robust phylogeny rather than in the context of the available chaotic traditional classifications.

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