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journal homepage: [www.elsevier.com/locate/revpalbo](http://www.elsevier.com/locate/revpalbo)Pollen morphology of alpine butterworts (*Pinguicula* L., Lentibulariaceae)

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## ABSTRACT

The pollen morphology of *Pinguicula alpina*, *P. arvetii*, *P. grandiflora* subsp. *grandiflora*, *P. grandiflora* subsp. *rosea*, *P. hirtiflora*, *P. leptoceras*, *P. poldinii*, *P. reichenbachiana*, and *P. vulgaris*, belonging to the Alpine flora, was studied.

The pollen grains, coming from different populations, were investigated using light microscopy and scanning electron microscopy. The pollen size, the shape (P/E ratio), the number of colpi and the exine ornamentation are, for *Pinguicula*, important diagnostic characters.

*Pinguicula* pollen grains are medium sized (~30 µm), trinucleate, isopolar, radially symmetric. The shape of the grains is variable from oblate spheroidal to prolate spheroidal and they are (4)–5–9–(10)-zonocolporate. The prevalent ornamentation is rugulate–microreticulate, *P. alpina* has a rugulate–reticulate ornamentation and only *P. hirtiflora* has a perforate ornamentation.

A pollen key, based on micromorphological data, is presented.

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## 1. Introduction

*Pinguicula* L., the second largest genus of the family Lentibulariaceae, contains about 100 currently accepted species. They are distributed in arctic, temperate, mediterranean and tropical areas of Eurasia, North America (Mexico has the largest number of species = 44; Zamudio, 2005), Central America, South America and Africa only in the northernmost region of Morocco (Zamora et al., 1996; Steiger, 1998; Degtjareva et al., 2004, 2006; Cieslak et al., 2005). In Europe, 12 species were reported by Casper (1972), but this number was expected to change due to the description of new species; the most recent were described in Italy (Tammaro and Pace, 1987; Casper and Steiger, 2001; Conti and Peruzzi, 2006; Ansaldo and Casper, 2009) and in Spain (Blanca et al., 1999). Alpine species are *P. alpina* L., *P. arvetii* Genty (endemic of Cozie Alps), *P. grandiflora* Lam. subsp. *grandiflora*, *P. grandiflora* subsp. *rosea* (Mutel) Casper (endemic in the calcareous mountains near Grenoble, France), *P. leptoceras* Rchb., *P. reichenbachiana* Schindler (endemic of Maritime Alps) and *P. vulgaris* L. (Casper, 1966; Pignatti, 1982; Aeschimann et al., 2004; Pascal et al., 2008; Compostella et al., 2010). *P. hirtiflora* Ten. is reported by Aeschimann et al. (2004) and only one population is known in Roya Valley, France: this population is considered to have been introduced by man because it is out of the typical area of the species (Central–Eastern Mediterranean according to Casper, 1966) (Peruzzi et al., 2004; Steiger and Tassara, 2006). Recently Casper and Steiger (2001)

described *P. poldinii* Steiger et Casper, a new endemic species in the hilly region of North–Eastern Venetian Prealps, Italy.

Alpine *Pinguicula* are herbaceous perennial insectivorous plants having leaves in a basal rosette, zygomorphic flowers and capsular fruits. They grow in nutrient-poor and partially sunny wet habitats: mainly vertical dripping limestone cliffs (*Cratoneurion* plant communities often involved in the processes of the travertine formation), hydromorphic alpine meadows but also banks of oligotrophic marshes and acidic *Sphagnum* bogs. The geographical distribution, for all the species of the genus, is highly fragmented due to the peculiarity and rarity of these habitats.

The published data on the pollen morphology of butterworts are still few, old, incomplete and based mainly on light microscopy observations. We focused on previous papers about the same species even if studied in different areas of the world. Concerning the alpine species, the pollen grains of *P. vulgaris* (but also *P. alpina* and *P. villosa*) are polycolporate (number of colpi 6–8), prolate spheroidal (39 × 37 µm), the exine is 2 µm thick and the sexine is finely reticulate (Erdtman et al., 1961). *P. vulgaris* was studied also by Sohma (1975) and Moore et al. (1991). Morphological data about *P. grandiflora* pollen grains were provided for the first time by Heslop-Harrison (2004) which reported a similarity with the ones of *P. vulgaris*. Recently Tsybalyuk et al. (2008) described, using LM and SEM, the pollen grains of Ukrainian populations of *P. vulgaris* and *P. alpina*. *P. alpina* is also reported by Hesse et al. (2009). Albanian populations of *P. hirtiflora* (var. *hirtiflora* and var. *louisii*) have been studied by Shuka et al. (2007) where a slight difference between the pollen grains of the two varieties was noticed.

Fossil pollen of *Pinguicula* has been recorded only by Mitchell (1954).

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Aim of this study is: a) to increase the knowledge about the pollen morphology of butterworts belonging to the Alpine Region, b) to compare our palynological data with those of previous authors, c) to give a pollen key based on micromorphological and quantitative data resulting from the study of different populations of alpine butterworts, d) to provide a valuable tool for pollen diagrams.

## 2. Materials and methods

Pollen grains of 9 taxa of the genus *Pinguicula* (belonging to the flora of the Alps) were studied. Specimens of different populations were collected in the field (Table 1; Fig. 1) during springs 2003–2008 and identified according to Casper (1966, 1974), Pignatti (1982), Casper and Steiger (2001), Aeschmann et al. (2004) and Pascal et al. (2008). Only flowers at the anthesis were picked up for a total of ~10 flowers for each population.

The *exsiccata* are housed in the herbarium of the Department of Biology, University of Milano: *Herbarium Universitatis Mediolanensis* (MI).

The palynological terminology used is according to Punt et al. (2007) and Hesse et al. (2009).

### 2.1. Light microscopy (LM)

Only flowers with mature anthers were used. Some pollen grains, removed from the anthers, were hydrated with distilled water on filter paper as in Rodondi et al. (2004), fixed in 2.5% glutaraldehyde, and stained with 4,6-diamidino-2-phenylindole (DAPI) dissolved in McIlvain buffer to detect DNA (Vergne et al., 1987). The observations were made with a LEICA DM-RD epifluorescent microscope equipped with a Ploëmopack 1 System and a mercury lamp (OSRAM HBO 100 W) with an excitation filter 340–380 nm, a dichroic beam-splitting mirror RKP 400, and a barrier filter at 420 nm. For the palynological studies, pollen grains were acetolyzed according to Erdtman (1960) and then concentrated and purified by using a density gradient of sucrose pads (Chissoe and Skvarla, 1974). The measures of P (= polar axis) and E (= equatorial diameter) of the pollen grains, mounted in glycerine jelly, were taken at 100× with a Leitz Wetzlar (Germany) microscope. The mean of P and E and the ratio P/E, useful to determine the shape, were obtained from the data based on measurements of 50 pollen grains for each population. The data elaboration is reported in Table 2.

### 2.2. Scanning electron microscopy (SEM)

Acetolyzed pollen, concentrated and purified according to Chissoe and Skvarla (1974) or stained in a sequence of osmium (O) and thiocarbonylhydrazide (T) solutions known as OTOTO method (Chissoe et al., 1995), was observed using a Leo 1430 and a Cambridge Stereoscan 360 scanning electron microscopes.

## 3. Results

### 3.1. LM survey (Plate I; Table 2)

The mature pollen grains of the investigated species of *Pinguicula* are released as free monads of medium size (~30 µm) (Plate I, 1–8). The pollen colour varies from whitish to yellow. Each grain is trinucleate (3-celled) (Plate I, 1).

Moreover, the grains are isopolar, stephanoaperturate, zonocolporate. Each aperture is associated with a colpus that is perpendicular to the equator and a bridge or constriction which seems to divide the colpus into two parts is often visible (Plate I, 3, 7). The number of colpi varies from species to species with a minimum of (4)–5–(6) colpi in *P. grandiflora* subsp. *rosea*, and a maximum of 8–9–(10) colpi in *P. hirtiflora*. Moreover the two subspecies of *P. grandiflora* show a different shape and number of colpi: suboblate and (5)–6–7–(8) colpi in subsp. *grandiflora* (Plate I, 2); oblate spheroidal and (4)–5–(6) colpi in subsp. *rosea* (Plate I, 3). Pollen grains of *P. poldinii* show a large number of anastomosing colpi. Around 60%, of the approximately 600 grains observed, has this characteristic feature, which makes these grains asymmetric and anomalous (Plate I, 7). The pollen grains of *Pinguicula* in polar view have a circular equatorial outline and the shape is oblate spheroidal. In *P. reichenbachiana* prolate pollen grains are the most frequent (Plate I, 8).

### 3.2. SEM survey (Plates II–V; Table 2)

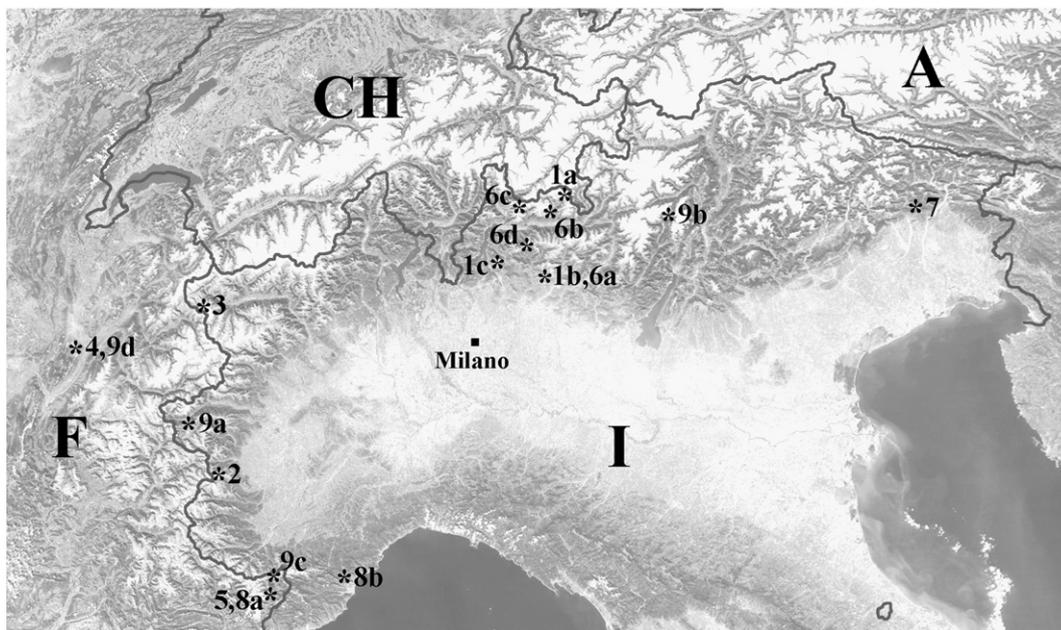
Each grain is stephanocolporate and its pores have a rectangular profile (Plate IV, 5; Plate V, 5, 9). The colpus membrane is often covered with granular elements (Plate III; 4) and the colpi are not normally fused at the polar edge. The inner structure of the sporoderm of some broken pollen grains was observed: there are two patterns of exine, one with a nearly continuous tectum like in *P. hirtiflora*, the other with a discontinuous tectum like in *P. reichenbachiana* (Plate II, 1–2).

**Table 1**  
Sampling and ecology of the examined taxa.

Species	Site (see also Fig. 1)	Elevation (m a.s.l.)	Habitat
<i>P. alpina</i> L.	1a – Alpe Gera (SO, Italy)	2100	Neutrophyle alpine grasslands
	1b – Monte Alben (BG, Italy)	1500	Carex firma, hydromorphic alpine grasslands, NE slope
	1c – Grigna Settentrionale (LC, Italy)	1600	Carex firma, hydromorphic alpine grasslands, SE slope
<i>P. arvetii</i> Genty	2 – Pian del Re, Monviso, Crissolo (CN, Italy)	2020	Acidophylous bog
<i>P. grandiflora</i> Lam. subsp. <i>grandiflora</i> <sup>a</sup>	3 – Rutor Valley (AO, Italy)	2135	Alpine heath
<i>P. grandiflora</i> subsp. <i>rosea</i> (Mutel) Casper	4 – Chapareillan (Département Isère, France)	1000	Wet banks of a sandy stream
<i>P. hirtiflora</i> Ten.	5 – Fontan, Val Roya (Département Alpes-Maritimes, France)	520	Dripping limestone cliffs
<i>P. leptoceras</i> Rchb.	6a – Monte Alben (BG, Italy)	1500	Carex firma, hydromorphic alpine grasslands, SE slope
	6b – Alpe Lago, Chiesa Val Malenco (SO, Italy)	1620	Marshes
	6c – Val Porcellizzo, Val Masino (SO, Italy)	1800	Wet acidophylous grasslands
	6d – Piano dell'acqua nera, Passo S. Marco (BG, Italy)	1750	Acidophylous bog
	7 – Campone, Tramonti di Sotto (PN, Italy)	450	Dripping limestone cliffs
	8a – Fontan, Val Roya (Département Alpes-Maritimes, France)	520	Dripping limestone cliffs
<i>P. poldinii</i> Steiger et Casper	8b – Villaggio Rocca Barbena (SV, Italy)	800	Dripping limestone cliffs
	9a – Sauze D'Oulx (TO, Italy)	1500	Wet meadows
<i>P. reichenbachiana</i> Schindl. <sup>b</sup>	9b – Passo Campo Carlo Magno, Pinzolo (TN, Italy)	1620	Wet meadows
	9c – Col de Tende (Département Alpes-Maritimes, France)	1250	Dripping limestone cliffs
	9d – Chapareillan (Département Isère, France)	1000	Wet meadows
	<i>P. vulgaris</i> L.		

<sup>a</sup> As *P. grandiflora* Lam. subsp. *grandiflora* f. *grandiflora*.

<sup>b</sup> As *P. longifolia* Ram. var. *reichenbachiana* (Schindler) Rouy or *P. longifolia* subsp. *reichenbachiana* (Schindler) Casper.



**Fig. 1.** Map of sites of the investigated species (see also Table 1): 1 (a, b, c) = *P. alpina*; 2 = *P. arvetii*; 3 = *P. grandiflora* subsp. *grandiflora*; 4 = *P. grandiflora* subsp. *rosea*; 5 = *P. hirtiflora*; 6 (a, b, c, d) = *P. leptoceras*; 7 = *P. poldinii*; 8 (a, b) = *P. reichenbachiana*; 9 (a, b, c, d) = *P. vulgaris*.

The exine ornamentation of *P. hirtiflora* is perforate (Plate IV, 4), while the ornamentation of all the other investigated species is rugulate–microreticulate or rugulate–reticulate (*P. alpina*) (Plate III, 2, 4, 6; Plate IV, 2, 6; Plate V, 3, 6, 9).

### 3.2.1. *P. alpina* (Plate III, 1–2)

The grains are suboblate, radially symmetric, isopolar and zonocolporate with (5)–6–7–(8) colpi. Rare anastomoses of colpi apices are visible. The profile of colpus margin is irregular and there are granules on the colpus membrane. The sexine ornamentation is rugulate–reticulate with thick curved muri (~1.0 µm thick), irregular narrow lumina (often wider than 1.0 µm) and rare perforations on the mesocolpium margin. There is usually no differentiation of the sexine towards the poles and/or colpi.

### 3.2.2. *P. arvetii* (Plate III, 3–4)

The grains are oblate spheroidal, radially symmetric, isopolar and zonocolporate with 5–6–(7) colpi. Anastomoses of colpi between the polar margins are very rare. The sexine ornamentation is rugulate–microreticulate, near the margin of the colpi, the muri merge and lumina become smaller, resembling perforations. Lumina are very variable in size and shape (rounded or polygonal) and they

are rarely wider than 1.0 µm. The muri are thick (thickness 0.5–1.0 µm).

### 3.2.3. *P. grandiflora* (Plate III, 5–6; Plate IV, 1–2)

In *P. grandiflora* the grains are suboblate in subsp. *grandiflora* and oblate spheroidal in subsp. *rosea*. The grains are radially symmetric, isopolar and zonocolporate with (5)–6–7–(8) colpi in subsp. *grandiflora*; (4)–5–(6) colpi in subsp. *rosea*. Rare anastomoses of colpi apices are visible. The sexine ornamentation is rugulate–microreticulate with thin curved muri (0.2–0.5 µm thick) and the lumina are very variable in size and shape (rarely wider than 1.0 µm). There is usually no differentiation of the sexine towards the poles and/or colpi.

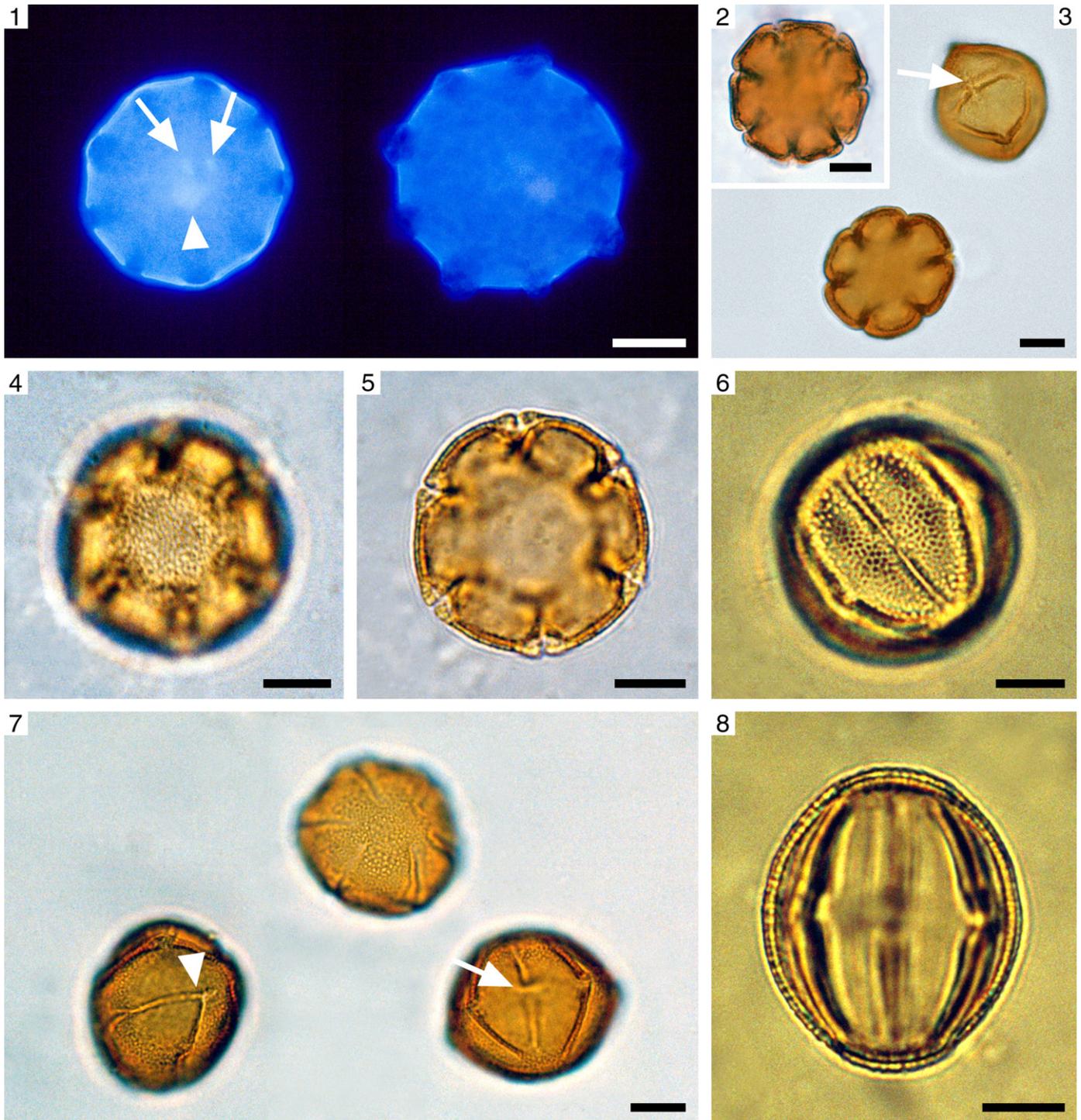
### 3.2.4. *P. hirtiflora* (Plate II, 1; Plate IV, 3–4)

The grains are oblate spheroidal, radially symmetric, isopolar, zonocolporate and parasyncolporate with 8–9–(10) colpi. The tectum is nearly continuous and perforated. Puncta (diameter <0.5 µm) have different shapes and sizes and their density on the mesocolpium margins and on the apocolpium is lower. The splitting of some pollen grains allowed observation of the thickness of the sporoderm layers: foot layer (0.2 µm), columellae (0.4 µm), tectum (0.4 µm). The whole thickness is around 1.0 µm.

**Table 2**

Biometric measures, based on 50 pollen grains for each population, of the examined taxa. Abbreviations: P = polar axis; E = equatorial diameter; SO = suboblate; OS = oblate spheroidal; PS = prolate spheroidal; S = spheroidal.

Species	P (µm)	E (µm)	P/E	Shape	Sexin ornamentation	Muri width (µm)	Number of colpi	
<i>P. alpina</i>	27.5 ± 0.15 (23.4–32.0)	32.1 ± 0.15 (28.8–36.0)	0.86	SO	Rugulate–reticulate	~1.0	(5)–6–7–(8)	
<i>P. arvetii</i>	28.5 ± 0.29 (24.3–32.9)	29.9 ± 0.28 (26.1–33.8)	0.96	OS	Rugulate–microreticulate	0.5–1.0	5–6–(7)	
<i>P. grandiflora</i>	subsp. <i>grandiflora</i>	26.9 ± 0.28 (23.4–31.5)	32.1 ± 0.29 (27.0–36.0)	0.84	SO	Rugulate–microreticulate	0.2–0.5	(5)–6–7–(8)
	subsp. <i>rosea</i>	28.6 ± 0.31 (24.3–33.3)	31.2 ± 0.29 (27.0–34.2)	0.92	OS	Rugulate–microreticulate	0.2–0.5	(4)–5–(6)
<i>P. hirtiflora</i>	29.4 ± 0.25 (25.2–33.3)	33.3 ± 0.26 (28.8–36.9)	0.88	OS	Perforate	–	8–9–(10)	
<i>P. leptoceras</i>	28.9 ± 0.28 (23.4–39.2)	31.1 ± 0.26 (26.1–41.0)	0.93	OS	Rugulate–microreticulate	0.5–1.0	5–6–(7)	
<i>P. poldinii</i>	31.3 ± 0.20 (27.0–34.2)	34.7 ± 0.27 (28.8–39.6)	0.90	OS	Rugulate–microreticulate	0.5–1.0	6–(7)	
<i>P. reichenbachiana</i>	30.2 ± 0.26 (23.4–35.1)	29.1 ± 0.23 (22.5–34.2)	1.04	PS	Rugulate–microreticulate	0.2–0.5	5–6	
<i>P. vulgaris</i>	32.3 ± 0.14 (27.0–37.8)	32.4 ± 0.13 (27.0–36.0)	1.00	S	Rugulate–microreticulate	0.5–1.0	6–7–(8)	



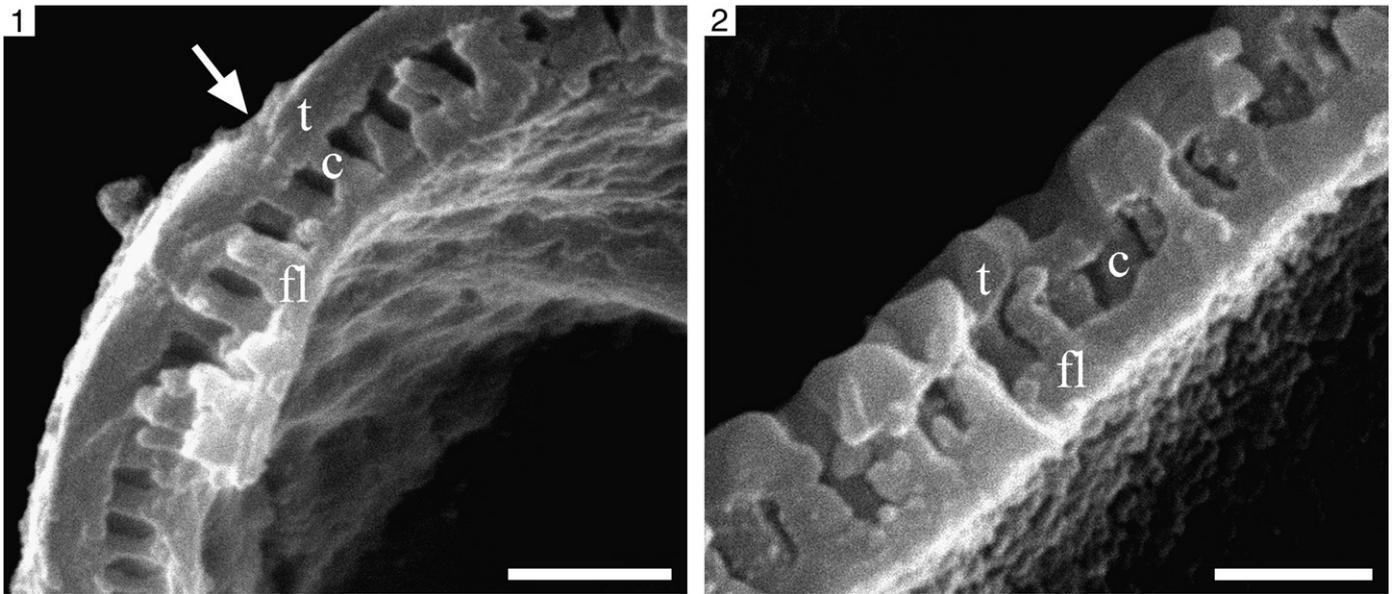
**Plate I.** LM micrographs (1 DAPI stain; 2–8 acetolyzed pollen). Scale bars = 10  $\mu$ m.

1. *P. hirtiflora*: pollen grains showing a vegetative nucleus (arrowhead) and two sperm nuclei (arrows), also 9 equatorial apertures are visible.
2. *P. grandiflora* subsp. *grandiflora*: polar view of a 7-colporate pollen grain.
3. *P. grandiflora* subsp. *rosea*: equatorial and polar view of pollen grains, an equatorial bridge is visible (arrow).
4. and 5. *P. alpina*: pollen grain in polar view, at two different focal planes, showing a reticulate ornamentation, colpi and the equatorial pores.
6. *P. reichenbachiana*: pollen grain in equatorial view showing a reticulate ornamentation.
7. *P. poldinii*: irregularly anastomosed colpi (arrowhead) are very frequent in this species. An equatorial bridge is also visible (arrow).
8. *P. reichenbachiana*: pollen grain in equatorial view with the typical prolate shape.

### 3.2.5. *P. leptoceras* (Plate IV, 5–6)

The grains are oblate spheroidal, radially symmetric, isopolar and zonocolporate with 5–6–(7) colpi. Anastomoses of colpi between

the polar margins are very rare. The sexine ornamentation is rugulate–microreticulate, near the margin of the colpi, the muri merge and lumina become smaller, resembling perforations. Lumina



**Plate II.** SEM micrographs of acetolyzed broken pollen grains (OTOTO-stained pollen). Abbreviations: t = tectum; c = columellae; fl = foot layer. Scale bars = 1 µm.

1. *P. hirtiflora*: pollen wall with a nearly continuous tectum a perforation is also visible (arrow).
2. *P. reichenbachiana*: pollen wall with discontinuous tectum.

are very variable in size and shape (rounded or polygonal) and they are rarely wider than 1.0 µm. The muri are thick (thickness 0.5–1.0 µm).

### 3.2.6. *P. poldinii* (Plate V, 1–3)

The grains are oblate spheroidal, radially symmetric or asymmetric, isopolar and zonocolporate with 6–(7) colpi. Many different types of anastomosing colpi are often visible on the grain. The sexine ornamentation is rugulate–microreticulate, near the margin of the colpi, the muri merge and lumina become smaller, resembling perforations. Lumina are very variable in size and shape (rarely wider than 1.0 µm) while the muri are thick (thickness 0.5–1.0 µm).

### 3.2.7. *P. reichenbachiana* (Plate II, 2; Plate V, 4–6)

The grains are prolate spheroidal, radially symmetric, isopolar and zonocolporate with 5–6 colpi and rare anastomoses close to the polar margins. The sexine ornamentation is rugulate–microreticulate, near the margin of the colpi, the muri merge and lumina become smaller resembling perforations. Lumina are very variable in size and their shape is often circular (rarely wider than 1.0 µm). The muri are thin (thickness 0.2–0.5 µm). The splitting of some pollen grains allowed observation of the thickness of the sporoderm layers: foot layer (0.4 µm), columellae (0.4 µm), tectum (0.5 µm). The whole thickness is around 1.3 µm.

### 3.2.8. *P. vulgaris* (Plate V, 7–9)

The grains are spheroidal, radially symmetric, isopolar and zonocolporate with 6–7–(8) colpi. Anastomoses between the polar margins are very rare. The sexine ornamentation is rugulate–microreticulate, near the margin of the colpi, the muri merge and lumina become smaller, resembling perforations. Lumina are very variable in size and shape (rarely wider than 1.0 µm). The muri are thick (thickness 0.5–1.0 µm).

Table 2 summarizes the main features.

## 4. Discussion

The institution of *Pinguicula poldinii* as a new species (Casper and Steiger, 2001), the discovery of new sites for *P. arvetii* (Pascal et al., 2008) and *P. grandiflora* subsp. *grandiflora* (Compostella et al., 2010) as new species of the Italian flora, together with the fact that the palynological data are still few, old and based mainly on light microscopy investigations, induced a methodical research on the micromorphology of the pollen grains of butterworts belonging to Alpine flora.

The comparative LM and SEM analysis of the pollen grains of the nine investigated taxa confirmed the general typology of the pollen grains of *Pinguicula* L. Detailed palynomorphological characteristics of the not yet investigated species *P. arvetii*, *P. grandiflora* (two subspecies), *P. leptoceras*, *P. poldinii*, *P. reichenbachiana* are provided for the first time.

In all the species of *Pinguicula* the pollen grains are released as monads which are isopolar, radially symmetric, stephanoperturate, zonocolporate and trinucleate (3-celled). The pollen colour, on fresh material, does not have any diagnostic relevance. The number of colpi is lower than 8, except in *P. hirtiflora* in which the number of colpi rises up to 10. The shape is from suboblate to oblate spheroidal ( $P < E$ ), except for *P. reichenbachiana*, that is prolate spheroidal ( $P > E$ ) (Table 2).

According to the pollen terminology (Punt et al., 2007; Hesse et al., 2009) the rugulate–microreticulate or rugulate–reticulate ornamentation characterizes the pollen wall of the butterworts investigated; only *P. hirtiflora* has a perforate ornamentation. *P. alpina* shows a rugulate–reticulate ornamentation characterized by very thick (around 1.0 µm) and twisted muri that make the lumina very narrow and irregular; the lumina sometimes are simple perforations. The microreticulum of *P. arvetii*, *P. leptoceras*, *P. poldinii* and *P. vulgaris* present thick muri (0.5–1.0 µm) in contrast with the thin muri (0.2–0.5 µm) of *P. grandiflora* (subsp. *grandiflora* and subsp. *rosea*) and *P. reichenbachiana*. The reticulum meshes of these last two species are larger than the one of the previous four species but nevertheless the ornamentation is still microreticulate. *P. grandiflora* and *P. reichenbachiana* differ because the first one has the muri more twisted and

its sexine ornamentation usually do not differentiate towards the poles and/or colpi. The microreticulate ornamentation of the mesocolpium margin (around 2.0 µm) changes from reticulate to perforate and the tectum varies from discontinuous to nearly continuous in almost all the taxa but not in *P. alpina* and *P. grandiflora* (subsp. *grandiflora* and subsp. *rosea*).

The perforate sexine ornamentation of *P. hirtiflora* shows a drastic reduction of puncta density near the margin of mesocolpium and on apocolpium.

The pollen of *P. poldinii* is very peculiar: around 60%, of the nearly 600 pollen grains observed, showed a large number of anastomoses between colpi and the grains are often asymmetric and anomalous. The anastomoses are very irregular and involve each part of the grain. Often it is impossible to distinguish between the polar and the equatorial region (Table 2); a similar feature has been reported also in *Utricularia breinii* Heer (Lentibulariaceae) (Huynh, 1968; Casper and Manitz, 1975; Käsermann and Moser, 1999). Casper and Steiger (2001) described briefly the pollen grains of *P. poldinii* and they found 6–8-colporate grains; even if we have never found 8-colporate grains we use this value to complete the proposed pollen key (Table 3).

The exine micromorphology by itself does not allow a clear separation of *P. arvetii*, *P. leptoceras*, *P. poldinii* and *P. vulgaris*. There are small differences regarding: the shape, the size and the number of colpi. Grains of *P. vulgaris* are spheroidal while they are oblate spheroidal in the other three species. Pollen grains of *P. vulgaris* and *P. poldinii* are larger than the ones of *P. arvetii* and *P. leptoceras*. 5-colporate pollen grains are frequent in *P. arvetii* and *P. leptoceras* while they have never been observed in *P. poldinii* and *P. vulgaris* (their minimum is 6-colporate) (Table 2). *P. arvetii* is so close to *P. leptoceras* that it is difficult to separate them properly using palynological data.

LM observations confirmed the general pollen morphology of *P. vulgaris* described by Sohma (1975) from Japanese herbarium specimens, and by Tsybalyuk et al. (2008) from Ukrainian herbarium specimens. The SEM observations of Sohma (1975) of *P. vulgaris*, described the ornamentation fairly irregular reticulate to rugulate, Tsybalyuk et al. (2008) describe a perforate ornamentation, while our data show a rugulate–microreticulate ornamentation. Also our data about the rugulate–reticulate sexine ornamentation of *P. alpina*

differs from Tsybalyuk et al. (2008) and Hesse et al. (2009) because they describe a micro-perforate ornamentation and an incomplete reticulum respectively.

The pollen grains of *P. vulgaris* and *P. grandiflora* from Alps are smaller than the one reported by Heslop-Harrison (2004) from English plants.

Finally a recent work of Shuka et al. (2007) exhaustively described two varieties of *P. hirtiflora* in Albania: var. *hirtiflora* and var. *louisii*. They described the grains stephano-7–9(–10) colpi, the shape from oblate to spheroidal and the ornamentation strongly microreticulate on equatorial mesocolpi and somewhat perforated on apocolpi in var. *hirtiflora*; microreticulate to vermiculate on equatorial mesocolpi in var. *louisii*. Our data cannot be properly compared with theirs because they worked on non-acetolyzed pollen grains. Be that as it may the ornamentation of our samples is always perforated and never microreticulate or vermiculate (as described in the Albanian varieties).

Our observations improve the knowledge on alpine butterworts and provide the diagnostic characters for the identification of their pollen grains. The diagnostic characters are: the size, the shape, the number of colpi and the exine ornamentation. These characters are combined to draw up a pollen key (Table 3) for the identification of the different species. This pollen key is an important tool for pollen diagrams and consequently for the reconstruction of paleo-wet oligotrophic-environments through the Holocene.

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**Plate III.** SEM micrographs of acetolyzed pollen grains. Scale bars: 1, 3, 5 = 10 µm; 2, 4, 6 = 1 µm. (see on page 7).

1. *P. alpina*: pollen grain in polar view.
2. *P. alpina*: rugulate-reticulate ornamentation with thick and twisted muri, and rare perforations (arrows).
3. *P. arvetii*: pollen grain in polar view.
4. *P. arvetii*: rugulate-microreticulate ornamentation with perforations (arrows) near the colpus margin and granular elements on the colpus membrane.
5. *P. grandiflora* subsp. *grandiflora*: pollen grain in polar view.
6. *P. grandiflora* subsp. *grandiflora*: rugulate–microreticulate ornamentation on apocolpium with thin and twisted muri; the reticulum has the same pattern even on mesocolpium margin.

**Plate IV.** SEM micrographs of acetolyzed pollen grains. Scale bars: 1, 3, 5 = 10 µm; 2, 4, 6 = 1 µm. (see on page 8)

1. *P. grandiflora* subsp. *rosea*: a 4-colporate pollen grain. Inset: the reticulum has the same pattern even on mesocolpium margin.
2. *P. grandiflora* subsp. *rosea*: rugulate–microreticulate ornamentation with thin and twisted muri.
3. *P. hirtiflora*: pollen grain in polar view.
4. *P. hirtiflora*: the number of perforations is reduced close to the apocolpium and the mesocolpium margin.
5. *P. leptoceras*: pollen grain in equatorial view showing a rectangular pore (arrowhead).
6. *P. leptoceras*: rugulate–microreticulate ornamentation with perforations (arrows) near the colpus margin.

**Plate V.** SEM micrographs of acetolyzed pollen grains. Scale bars: 1–2, 4, 7–8 = 10 µm; 3, 5–6, 9 = 1 µm. (see on page 9)

1. *P. poldinii*: pollen grains showing anastomosing colpi close to the polar margins (arrow).
2. *P. poldinii*: pollen grain with an extreme irregularity in its colpi.
3. *P. poldinii*: rugulate–microreticulate ornamentation.
4. *P. reichenbachiana*: pollen grain in equatorial view showing a bridge (arrow). The alteration of the ornamentation pattern on mesocolpium, near the colpus margin, is also visible.
5. *P. reichenbachiana*: perforations (arrows) near the colpus margin and a rectangular pore (arrowhead).
6. *P. reichenbachiana*: rugulate–microreticulate ornamentation with thin muri.
7. and 8. *P. vulgaris*: pollen grain in oblique equatorial view, showing a small difference in the size of the polar regions.
9. *P. vulgaris*: rugulate–microreticulate ornamentation with perforations (arrows) near the colpus margin and a rectangular pore (arrowhead).

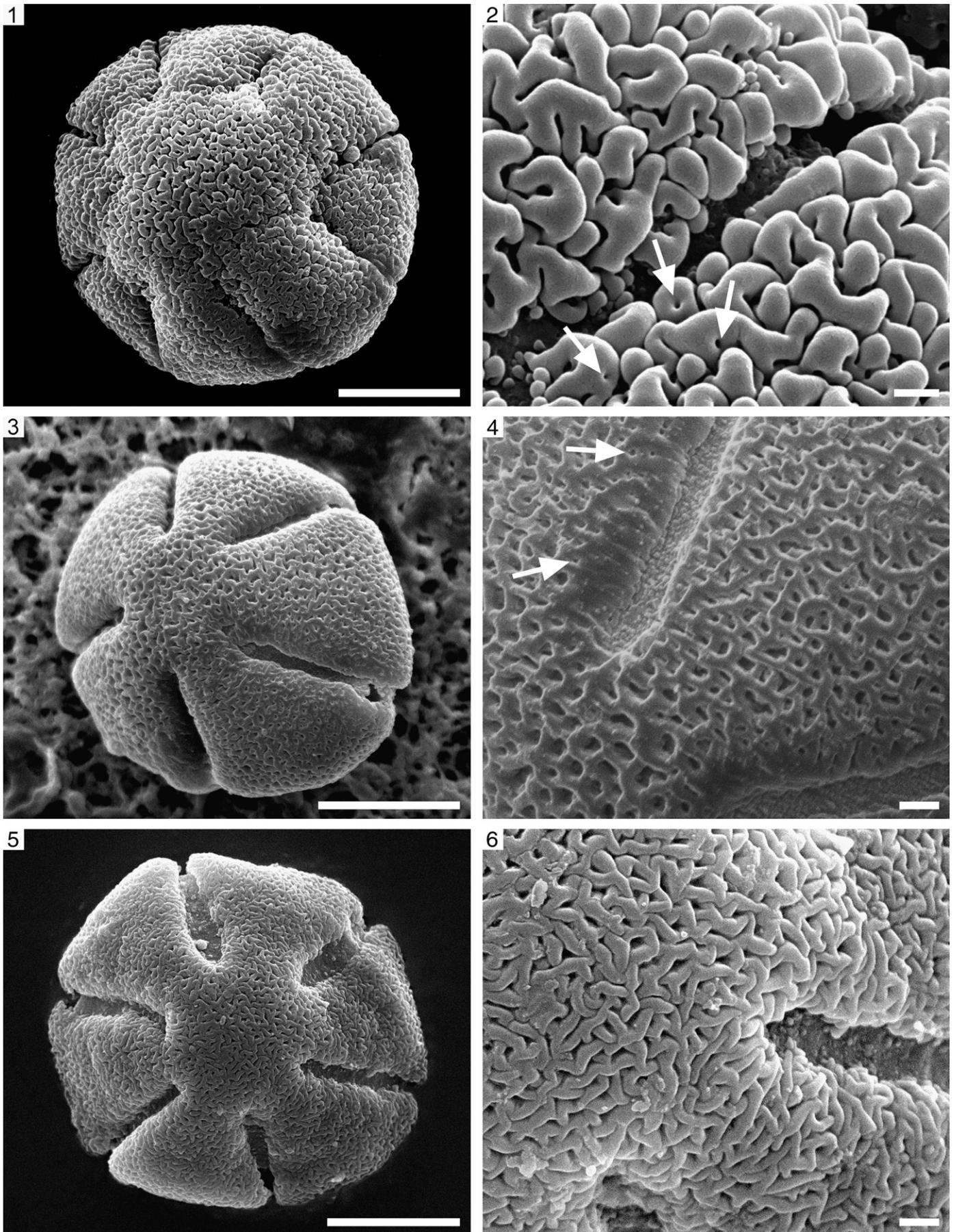


Plate III.

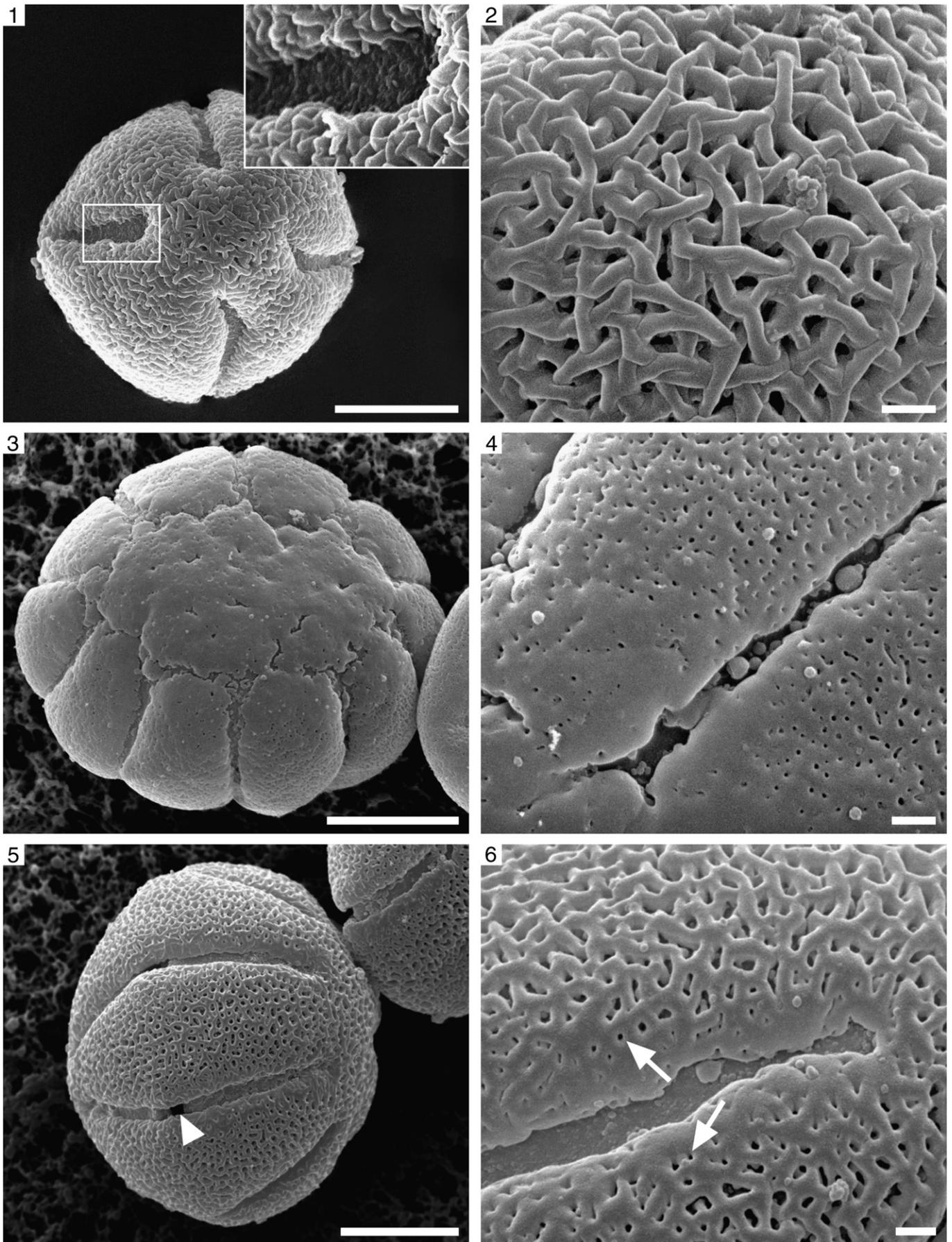


Plate IV (caption on p. 6).

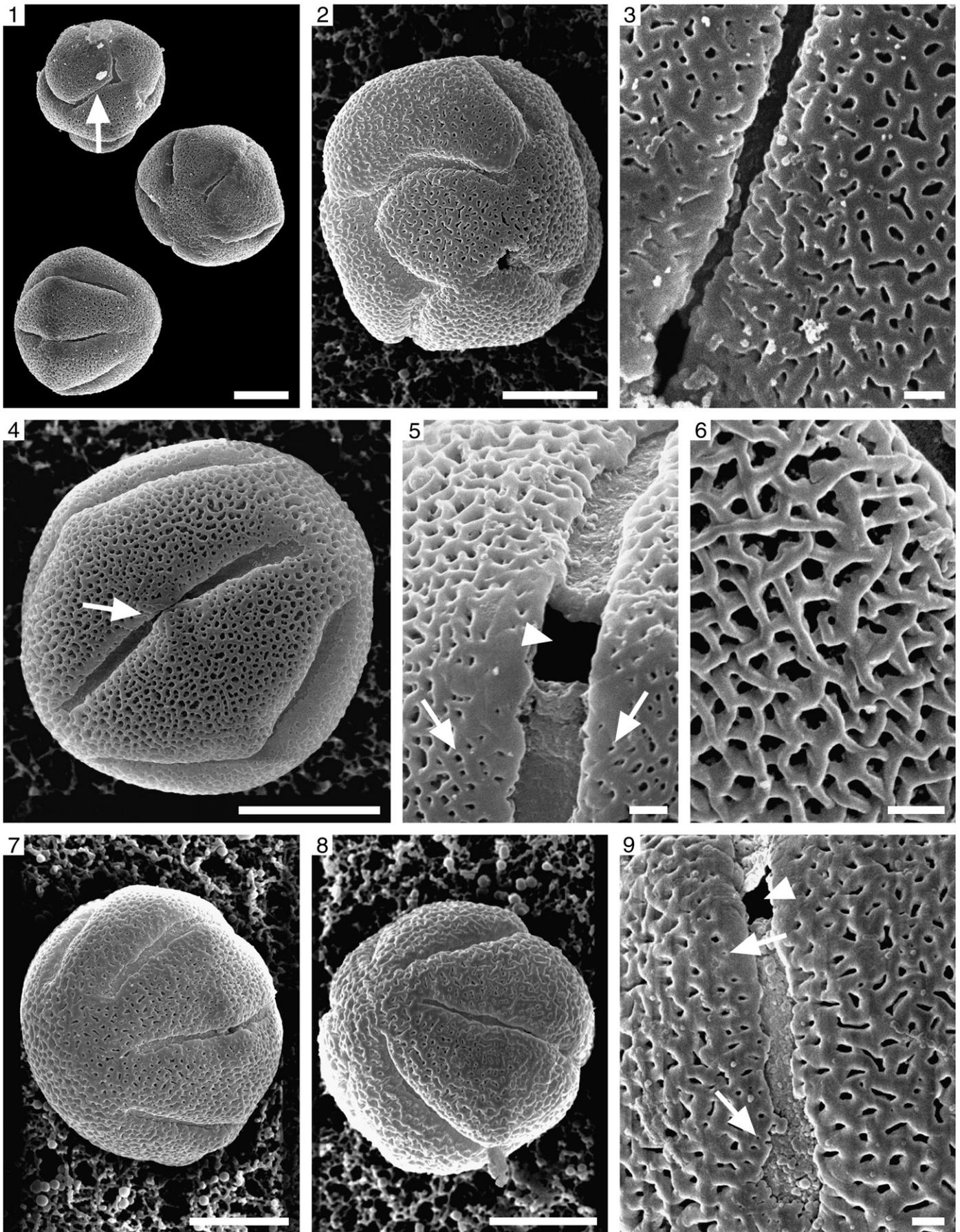


Plate V (caption on p. 6).

Table 3

Pollen key.

1. Tectum nearly continuous with a perforate ornamentation, number of colpi $\geq 8$	<i>P. hirtiflora</i>
1.* Tectum discontinuous with a rugulate–microreticulate or rugulate–reticulate ornamentation, number of colpi $\leq 8$	
2. Tectal muri thin: 0.2–0.5 $\mu\text{m}$ thick	
3a. Prolate spheroidal shape, rugulate–microreticulate ornamentation, 5–6 colpi	<i>P. reichenbachiana</i>
3b. Suboblate shape, rugulate–microreticulate ornamentation with twisted muri, (5)–6–7–(8) colpi	<i>P. grandiflora</i> subsp. <i>grandiflora</i>
3c. Oblate spheroidal shape, rugulate–microreticulate ornamentation with twisted muri, (4)–5–(6) colpi	<i>P. grandiflora</i> subsp. <i>rosea</i>
2.* Tectal muri thick: 0.5–1.0 $\mu\text{m}$ thick	
4. Suboblate shape, rugulate–reticulate ornamentation with compact and twisted muri (~1.0 $\mu\text{m}$ thick)	<i>P. alpina</i>
4.* Shape from oblate spheroidal to spheroidal, rugulate–microreticulate ornamentation with muri 0.5–1.0 $\mu\text{m}$ thick	
5. Spheroidal shape, P and E $> 30 \mu\text{m}$ , 6–7–(8) colpi	<i>P. vulgaris</i>
5.* Oblate spheroidal shape	
6a. P and E $< 30 \mu\text{m}$ , 5–6–(7) colpi	<i>P. arvetii</i>
6b. P $< 30 \mu\text{m}$ and E $> 30 \mu\text{m}$ , 5–6–(7) colpi	<i>P. leptoceras</i>
6c.* P and E $> 30 \mu\text{m}$ , 6–(7–8) <sup>a</sup> colpi. Asymmetric, anomalous pollen grains with frequent anastomosing colpi	<i>P. poldinii</i>

<sup>a</sup> Our data, 6(–7) colpi, combined with Casper and Steiger (2001), 6–8 colpi.

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