

Pollen morphology in *Saxifraga* (Saxifragaceae) from Ny-Ålesund, Svalbard, Arctic, and its taxonomic significance

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Abstract Pollen morphology of eight species of *Saxifraga*, i.e. *S. cespitosa*, *S. oppositifolia*, *S. cernua*, *S. nivalis*, *S. aizoides*, *S. rivularis*, *S. hieraciifolia*, and *S. hirculus*, collected from Ny-Ålesund, Svalbard, Arctic was examined using light and scanning electron microscopy. Pollen grains of *Saxifraga* species are subprolate or prolate, 3-colpate, 15.4–44.4 µm in the polar axis, 11.4–34.6 µm in the equatorial axis, and show a *P/E* ratio 1.19–1.40. On the basis of exine ornamentation, four pollen types, viz., the *S. oppositifolia* type (striate without scabrae on the muri), *S. cernua* type (striate with scabrae on the muri), *S. nivalis* type (microreticulate and operculum absent), and *S. cespitosa* type (microechinate and operculum present), were recognized. Hierarchical cluster analysis of the pollen morphological characters indicated that pollen morphology supports the infrageneric classification of the genus *Saxifraga*.

Keywords Arctic, Ny-Ålesund, pollen morphology, *Saxifraga*, Svalbard

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1 Introduction

Saxifraga is the largest genus in the family Saxifragaceae, containing approximately 440 species mostly distributed in northern temperate regions, especially Europe, Himalaya and East Asia, and northwestern America to the Arctic^[1]. Fourteen species are recorded in the Svalbard Archipelago, Arctic^[2]. The genus is characterized by a perennial, herbaceous habit, stem caespitose or simple, leaves both basal and cauline, 5-merous flowers usually bisexual, and capsular fruit^[3]. It is subdivided into 15 sections, viz., *Ciliatae*, *Cotylea*, *Cymbalaria*, *Gymnopera*, *Heterisia*, *Irregulares*, *Ligulatae*, *Merkianae*, *Mesogyne*, *Micranthes*, *Odontophyllae*, *Porphyron*, *Saxifraga*, *Trachyphyllum*, and *Xanthizoon*^[1,4].

Pollen morphology of the genus *Saxifraga* has been

studied by a number of palynologists^[5-14], of which few have used pollen morphological characters in the classification of the genus. The pollen morphology of *Saxifraga* species from Svalbard (74°–81°N, 10°–35°E), especially from Ny-Ålesund (78°55'N, 11°56'E), off the western coast of Svalbard, has not been reported previously. In the present study, we examined the pollen morphology of eight species of *Saxifraga* from Ny-Ålesund by means of light microscopy (LM) and scanning electron microscopy (SEM), and compiled a key to the species. In addition, a hierarchical cluster analysis was conducted in order to explore the taxonomic significance of pollen characters.

2 Materials and methods

Flowering specimens of eight *Saxifraga* species (Table

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1) were collected from Ny-Ålesund, Svalbard (Figure 1), during the Scientific Expedition of the Chinese Arctic Yellow River Station in July 2012. Pollen grains were extracted from anthers of the flowers and studied by LM and SEM. For LM studies, pollen grains were acetolyzed following Erdtman's^[15] method and mounted in stained glycerine jelly. The slides were observed under a Leica DM 2500 light microscope equipped with a 100× oil immersion lens and a 10× eyepiece. Pollen measurements were based on 20 individual grains selected randomly for each species. Polar axis diameter (*P*), equatorial diameter (*E*), and exine thickness were measured on micrometric slides under a magnification of 1 000×. The *P/E* values were calculated for each measured pollen grain. For SEM studies, pollen grains were dehydrated in an ethanol gradient series, dispersed on SEM stubs, and sputter-coated with gold using a Hitachi E-1010 sputter

coater. The samples were then examined and photographed under a Hitachi S-4800 scanning electron microscope at an accelerating voltage of 10 kV.

Table 1 Eight species of *Saxifraga* sampled from Ny-Ålesund, Svalbard

Species	Latitude & Longitude
<i>S. oppositifolia</i>	78°55'01.1" N, 11°56'56.0" E
<i>S. hirculus</i>	78°57'39.0" N, 11°35'53.0" E
<i>S. hieracifolia</i>	78°55'05.0" N, 11°56'31.0" E
<i>S. nivalis</i>	78°56'14.7" N, 11°51'10.3" E
<i>S. rivularis</i>	78°55'49.9" N, 11°50'34.9" E
<i>S. cernua</i>	78°55'01.1" N, 11°56'56.0" E
<i>S. cespitosa</i>	78°55'01.1" N, 11°56'56.0" E
<i>S. aizoides</i>	78°55'49.9" N, 11°50'34.9" E

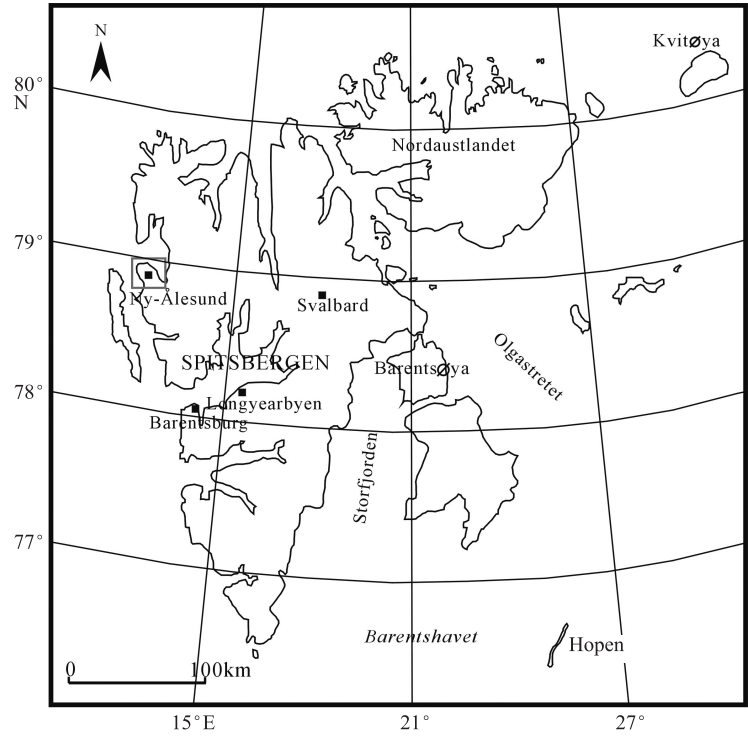


Figure 1 Map showing the location of Ny-Ålesund, Svalbard (indicated by square).

For pollen descriptive terminologies, Punt et al.^[16] and Hesse et al.^[17] are followed. Pollen size classes are used as proposed by Hesse et al.^[17], viz., very small (<10 μm), small (10–25 μm), medium (26–50 μm), large (51–100 μm), and very large (>100 μm). Shape classes in equatorial view (*P/E*) are adopted, i.e. suboblate (0.75–0.88), spheroidal (0.88–1.14), subprolate (1.14–1.33), and prolate (1.33–2.00) as proposed by Punt et al.^[16]

For hierarchical cluster analysis of the eight species of *Saxifraga*, seven variable pollen characters were used, consisting of five quantitative characters (polar axis, equatorial axis, *P/E*, volume index, and exine thickness) and two qualitative characters (pollen shape and exine ornamentation). The quantitative characters were standardized by square-

root transformation before the analysis, and the qualitative characters were coded in sequence. For example, pollen shape comprised two states, namely subprolate and prolate, which were coded as 1 and 2, respectively. After standardization and coding of the characters, SPSS Statistics 17.0 software was used to perform between-groups average linkage and measure Euclidean distances for the cluster analysis.

3 Results

3.1 Description of pollen grains in *Saxifraga*

Saxifraga oppositifolia L. (Figure 2(a)–(f), Table 2)
Pollen grains subprolate, 24.2 (21.2–27.2) μm × 18.8

(15.0–21.8) μm . P/E ratio 1.30. Elliptic in equatorial view and circular in polar view. Apertures 3-colpate. Colpus long, extending to the poles, rather broad, ends obtuse. Colpus margin regular or irregular. Colpus membrane and operculum sometimes absent. Exine ca. 1.8 μm thick. Sexine thicker than nexine. Ornamentation striate under LM and SEM, muri as wide as or wider than grooves, anastomosing.

Saxifraga hirculus L. (Figure 2(g)–(l), Table 2)

Pollen grains prolate, 25.9 (22.2–29.6) $\mu\text{m} \times 18.6$ (16.5–21.3) μm . P/E ratio 1.40. Elliptic in equatorial view and circular in polar view. Apertures 3-colpate. Colpus long, extending to the poles, broad, sunken, margin regular.

Operculum absent. Exine ca. 2.2 μm thick. Sexine thicker than nexine. Ornamentation striate under LM, striate with scabrae on the muri under SEM.

Saxifraga hieraciifolia Waldst. & Kit. (Figure 3(a)–(f), Table 2)

Pollen grains subprolate, 20.5 (17.4–24.3) $\mu\text{m} \times 16.1$ (14.2–18.2) μm . P/E ratio 1.30. Elliptic in equatorial view and circular in polar view. Apertures 3-colpate. Colpus long, extending to the poles, broad, sunken. Colpus margin distinct, membrane smooth. Operculum absent. Exine ca. 2.0 μm thick. Sexine almost equal to nexine in thickness. Ornamentation granulate-reticulate under LM, microreticulate

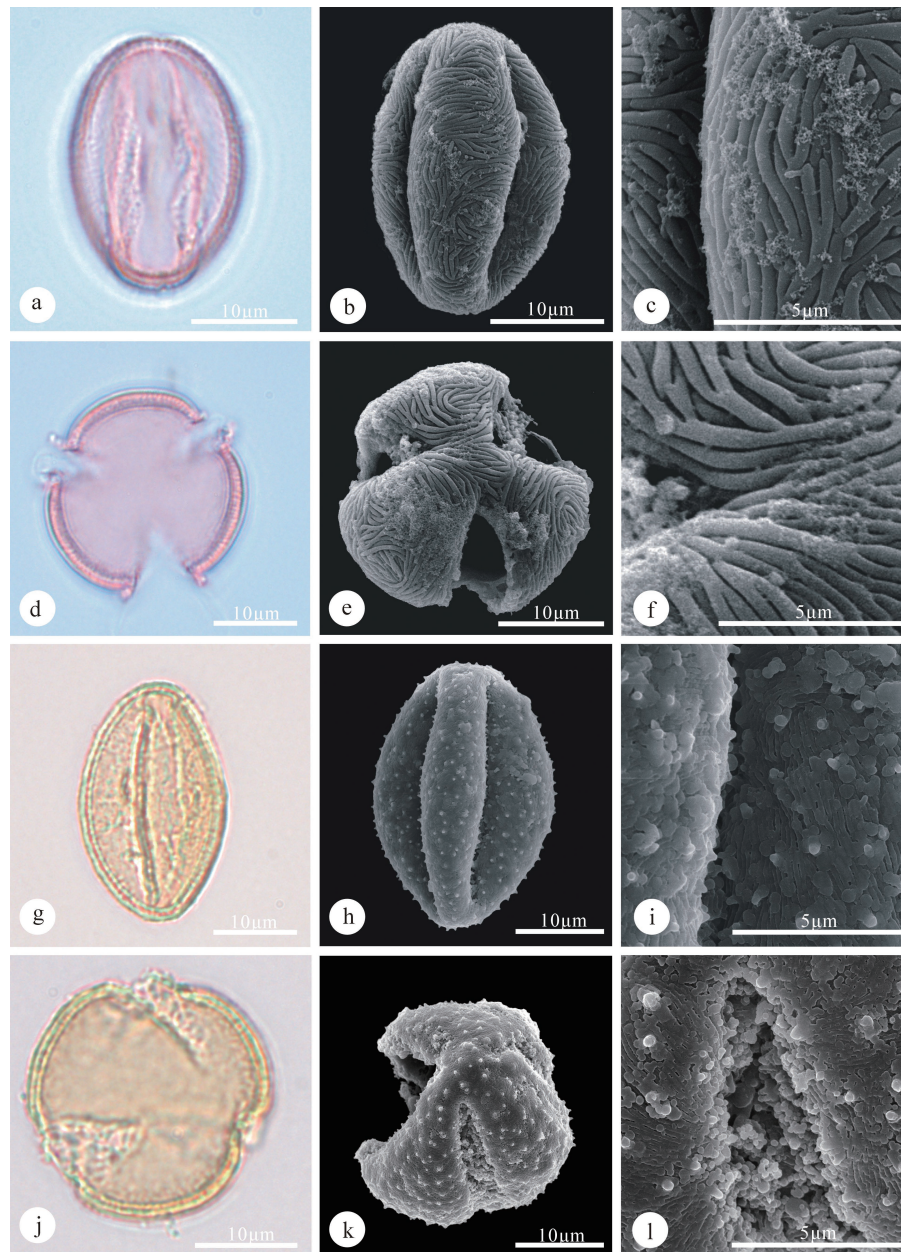


Figure 2 Pollen grains of *Saxifraga oppositifolia* (a–f) and *Saxifraga hirculus* (g–l). **a, g**: Equatorial view (LM); **b, h**: Equatorial view (SEM); **d, j**: Polar view (LM); **e, k**: Polar view (SEM); **c, f, i, l**: Exine ornamentation (SEM); **a, b, g, h, j**: Colpus membrane present but operculum absent; **d, e**: Both colpus membrane and operculum absent; **k**: One colpus membrane and operculum absent.

under SEM. Lumina variable in size and shape.

Saxifraga nivalis L. (Figure 3(g)–(l), Table 2)

Pollen grains subprolate, 18.8 (15.4–25.4) $\mu\text{m} \times 14.9$ (11.4–22.0) μm . *P/E* ratio 1.29. Elliptic in equatorial view and circular in polar view. Apertures 3-colpate. Colpus very long, extending to the poles, rather narrow, sunken, ends acute. Colpus margin distinct, membrane smooth. Operculum absent. Exine ca. 1.9 μm thick. Sexine thicker than nexine. Ornamentation granulate-reticulate under LM, microreticulate under SEM. Lumina variable in size and shape.

Saxifraga rivularis L. (Figure 4(a)–(f), Table 2)

Pollen grains subprolate, 26.1 (15.4–44.4) $\mu\text{m} \times 21.2$ (13.4–34.6) μm . *P/E* ratio 1.23. Elliptic in equatorial view

and circular in polar view. Apertures 3-colpate. Colpus long, extending to the poles, broad, sunken, margin regular. Operculum absent. Exine ca. 2.1 μm thick. Sexine thicker than nexine. Ornamentation striate under LM, striate with scabrae on the muri under SEM. Muri wider than the grooves.

Saxifraga cernua L. (Figure 4(g)–(l), Table 2)

Pollen grains subprolate, 27.4 (23.8–31.2) $\mu\text{m} \times 22.5$ (18.4–24.2) μm . *P/E* ratio 1.24. Elliptic in equatorial view and circular in polar view, outline sometimes undulate. Apertures 3-colpate. Colpus long, extending to the poles, rather narrow, sunken, ends acute, margin irregular, membrane with fine granules. Operculum absent. Exine ca. 2.3 μm thick. Sexine thicker than nexine. Ornamentation coarsely granulate under

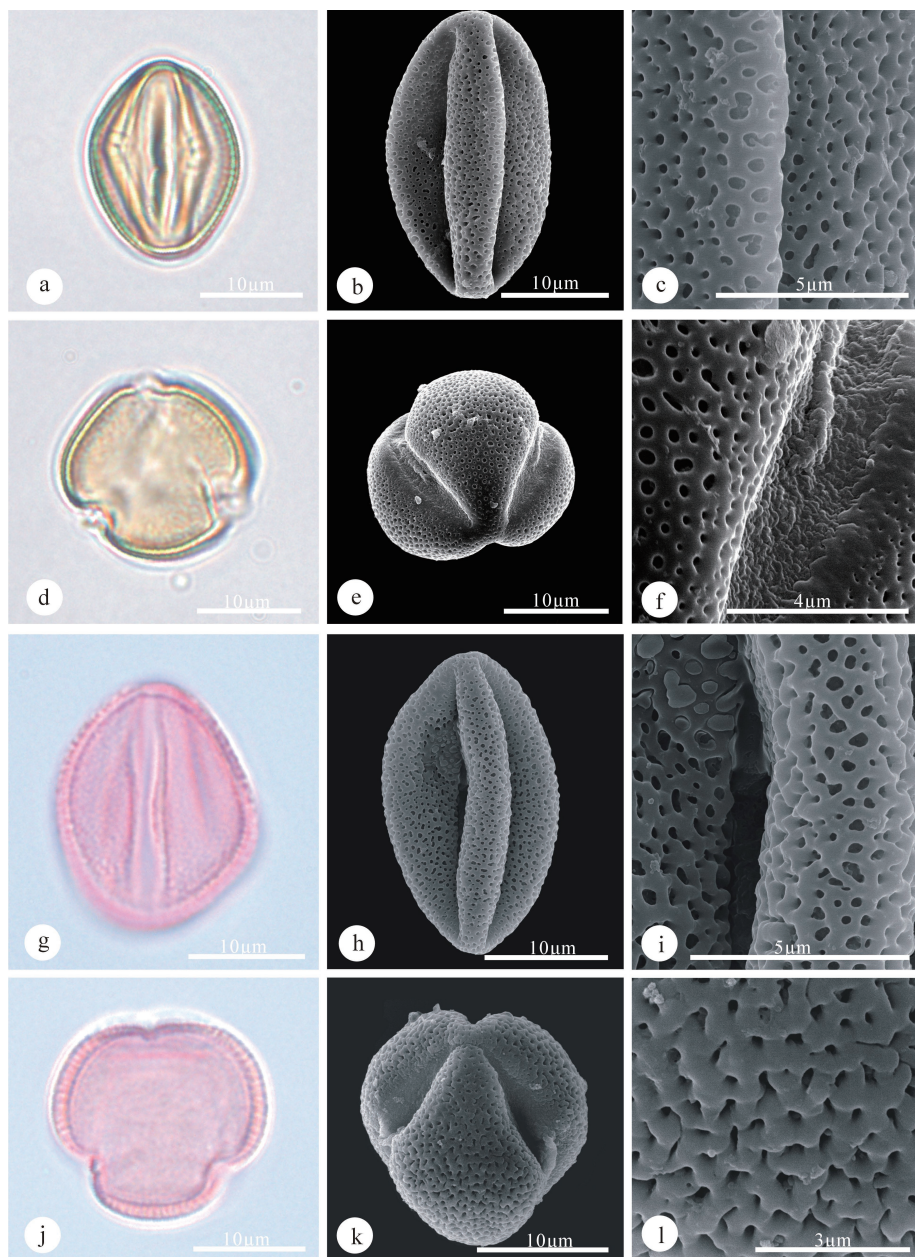


Figure 3 Pollen grains of *Saxifraga hieraciifolia* (a–f) and *Saxifraga nivalis* (g–l). **a, g**: Equatorial view (LM); **b, h**: Equatorial view (SEM); **d, j**: Polar view (LM); **e, k**: Polar view (SEM); **c, f, i, l**: Exine ornamentation (SEM); **a, b, d, e, g, h, j, k**: Colpus membrane present but operculum absent.

Table 2 Pollen characters of the eight species of *Saxifraga* sampled from Ny-Ålesund, Svalbard

Species	Pollen shape	Aperture	Polar axis/ μm	Equatorial axis/ μm	P/E	Exine ornamentation	Volume index	Exine thickness/ μm	Figure
<i>S. oppositifolia</i>	subprolate	3-colpate	(21.2-) 24.2 (-27.2)	(15.0-) 18.8 (-21.8)	1.30	striate without scabrae	21.3	1.8	2(a)-(f)
<i>S. hirculus</i>	prolate	3-colpate	(22.2-) 25.9 (-29.6)	(16.5-) 18.6 (-21.3)	1.40	striate with scabrae	21.9	2.2	2(g)-(l)
<i>S. hieraciifolia</i>	subprolate	3-colpate	(17.4-) 20.5 (-24.3)	(14.2-) 16.1 (-18.2)	1.30	microreticulate	18.2	2.0	3(a)-(f)
<i>S. nivalis</i>	subprolate	3-colpate	(15.4-) 18.8 (-25.4)	(11.4-) 14.9 (-22.0)	1.29	microreticulate	16.7	1.9	3(g)-(l)
<i>S. rivularis</i>	subprolate	3-colpate	(15.4-) 26.1 (-44.4)	(13.4-) 21.2 (-34.6)	1.23	striate with scabrae	23.5	2.1	4(a)-(f)
<i>S. cernua</i>	subprolate	3-colpate	(23.8-) 27.4 (-31.2)	(18.4-) 22.5 (-24.2)	1.24	striate with scabrae	24.8	2.3	4(g)-(l)
<i>S. cespitosa</i>	subprolate	3-colpate	(24.4-) 26.3 (-29.2)	(17.6-) 21.9 (-25.6)	1.20	microechinate	24.0	1.5	5(a)-(f)
<i>S. aizoides</i>	subprolate	3-colpate	(15.8-) 20.3 (-24.0)	(11.4-) 17.3 (-22.6)	1.19	striate with scabrae	18.7	2.3	5(g)-(l)

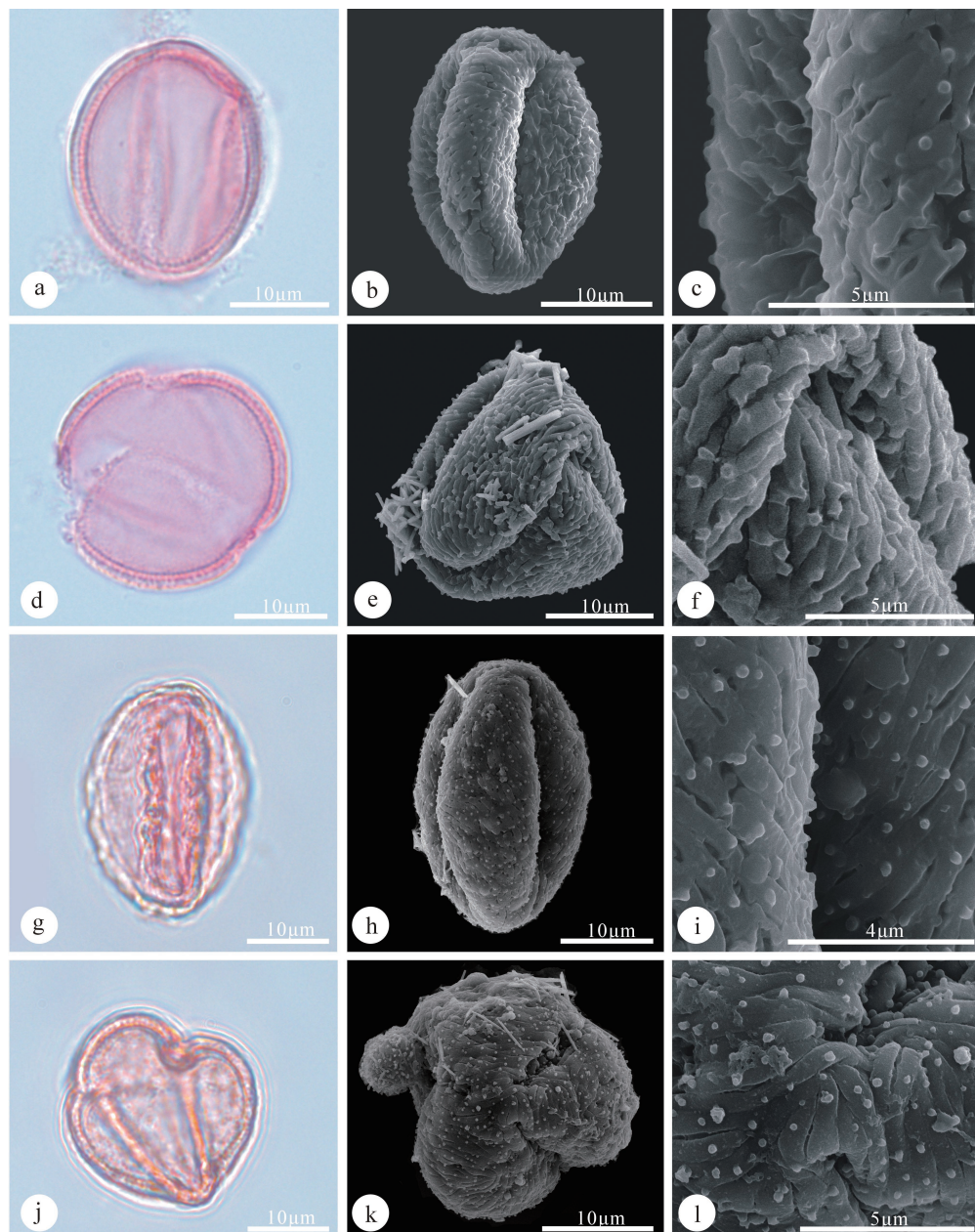


Figure 4 Pollen grains of *Saxifraga rivularis* (a–f) and *Saxifraga cernua* (g–l). **a, g**: Equatorial view (LM); **b, h**: Equatorial view (SEM); **d, j**: Polar view (LM); **e, k**: Polar view (SEM); **c, f, i, l**: Exine ornamentation (SEM); **a, b, d, e, g, h, j, k**: Colpus membrane present but operculum absent.

LM, striate with scabrae on the muri under SEM.

Saxifraga cespitosa L. (Figure 5(a)–(f), Table 2)

Pollen grains subprolate, 26.3 (24.4–29.2) $\mu\text{m} \times 21.9$ (17.6–25.6) μm . *P/E* ratio 1.20. Elliptic in equatorial view and circular in polar view. Apertures 3-colpate. Colpus long, extending to the poles, rather broad, sunken, sometimes deeply, ends acute. Colpus margin regular, membrane usually microechinate. Operculum present. Exine ca. 1.5 μm thick. Sexine thicker than nexine. Ornamentation granulate under LM, microechinate under SEM.

Saxifraga aizoides L. (Figure 5(g)–(l), Table 2)

Pollen grains subprolate, 20.3 (15.8–24.0) $\mu\text{m} \times 17.3$ (11.4–22.6) μm . *P/E* ratio 1.19. Elliptic in equatorial view and circular in polar view, outline bisymmetric, with one side obtuse and the other side convex. Apertures 3-colpate. Colpus long, extending to the poles, narrow, sunken deeply, ends acute, margin regular. Operculum usually absent. Exine ca. 2.3 μm thick. Sexine about twice as thick as the nexine. Ornamentation striate under LM, striate with scabrae on the

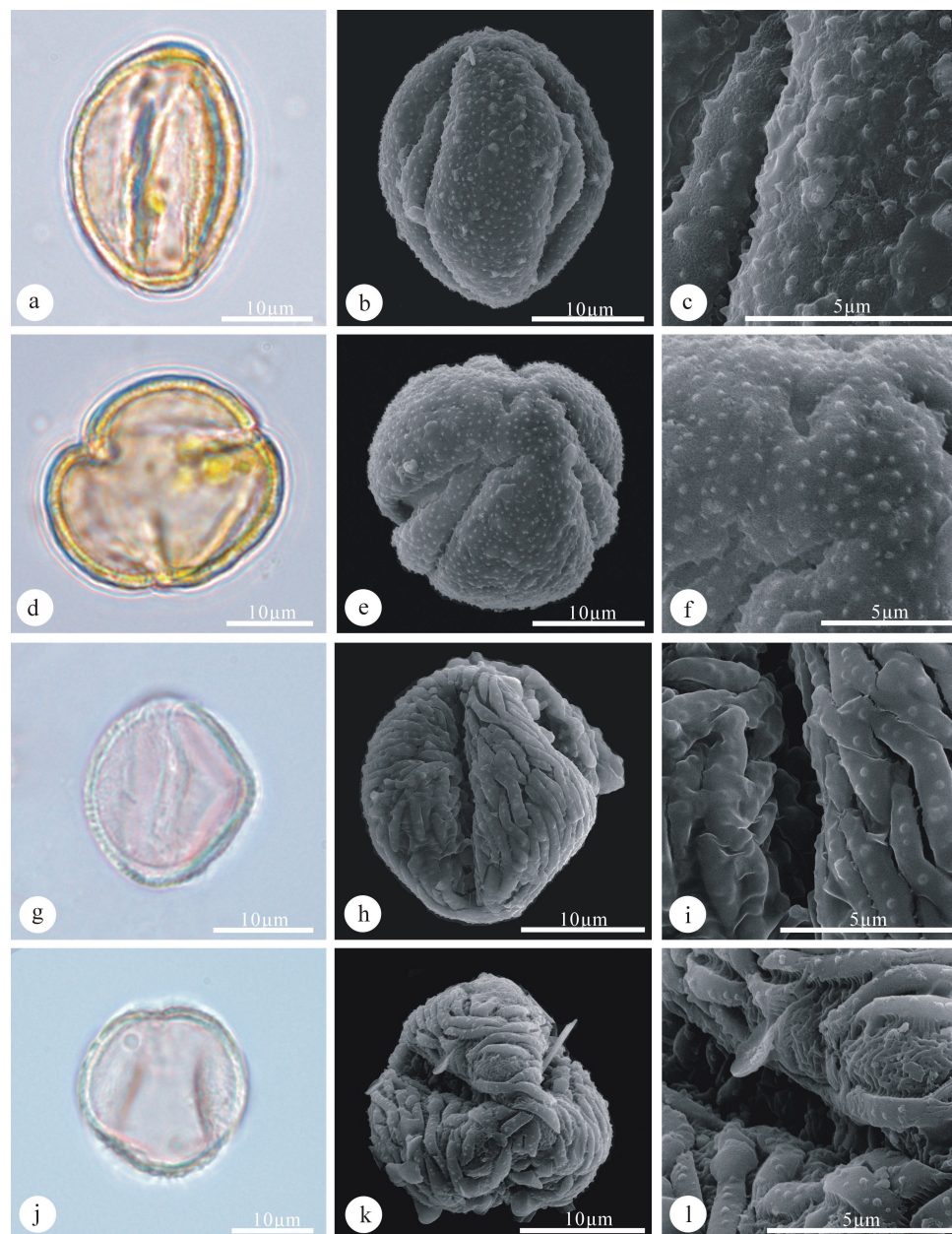


Figure 5 Pollen grains of *Saxifraga cespitosa* (a–f) and *Saxifraga aizoides* (g–l). a, g: Equatorial view (LM); b, h: Equatorial view (SEM); d, j: Polar view (LM); e, k: Polar view (SEM); c, f, i, l: Exine ornamentation (SEM); a, b, d, e: Both colpus membrane and operculum present; g, h, j, k: Colpus membrane present but operculum absent.

muri under SEM. Muri wider than the grooves.

Key to the species

1. a. Pollen grains subprolate, exine ornamentation microreticulate or microechinate.....2
- b. Pollen grains subprolate or prolate, exine ornamentation striate.....3
2. a. Exine ornamentation microreticulate, operculum absent.....*S. nivalis*, *S. hieraciifolia*
- b. Exine ornamentation microechinate, operculum present.....*S. cespitosa*
3. a. Pollen grains subprolate, exine ornamentation striate without scabrae on muri.....*S. oppositifolia*
- b. Pollen grains subprolate or prolate, exine ornamentation striate with scabrae on muri.....4
4. a. Pollen grains prolate, *P/E* approximately 1.40.....*S. hirculus*
- b. Pollen grains subprolate, *P/E* approximately5
5. a. Polar axis 20.3 μm and equatorial axis 17.3 μm on average.....*S. aizoides*
- b. Polar axis 26–27 μm and equatorial axis 21–23 μm on average.....*S. cernua*, *S. rivularis*

3.2 Hierarchical cluster analysis

The dendrogram showed that the eight species of *Saxifraga* were divided into two groups (Figure 6). Group 1 comprised five species with striate ornamentation of the exine, viz., *S. cernua*, *S. rivularis* (Section *Mesogyne*), *S. aizoides* (Section *Xanthizoon*), *S. hirculus* (Section *Ciliatae*), and *S. oppositifolia* (Section *Porphyron*). The former four species have striae with scabrae on the muri, whereas the latter species has striae without scabrae. *Saxifraga cernua* and *S. rivularis* were more closely related than with the other species. Group 2 included three species with microreticulate and microechinate sculptures, viz., *S. nivalis*, *S. hieraciifolia* (Section *Micranthes*), and *S. cespitosa* (Section *Saxifraga*). The relationships among the different species revealed by cluster analysis of pollen morphological characters were generally consistent with other morphological characteristics. For example, *S. cernua* and *S. rivularis* have kidney-shaped basal leaves and white flowers, *S. nivalis* and *S. hieraciifolia* have a single, stout stem and toothed leaves. *Saxifraga hirculus* is distinguished from the other species by lanceolate, glabrous basal leaves and yellow flowers^[2].

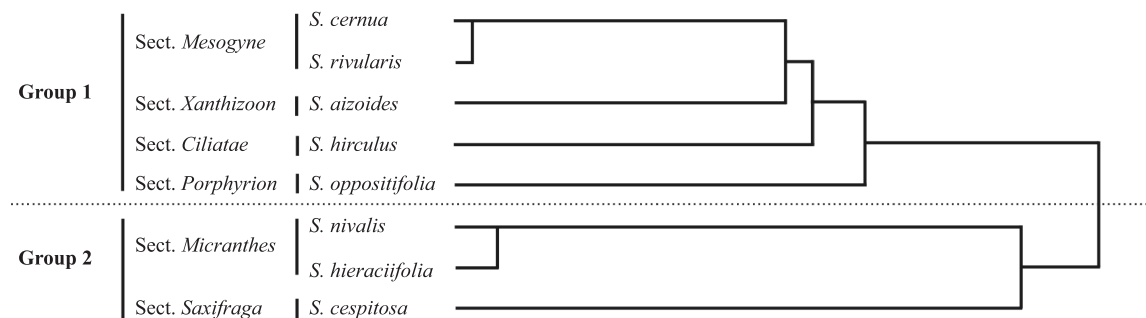


Figure 6 Hierarchical clustering dendrogram representing relationships among the eight species of *Saxifraga* on the basis of pollen morphological characters.

4 Discussion

The pollen morphology of the genus *Saxifraga* comprises a number of variable and invariable characters. In the eight investigated species from Ny-Ålesund, Svalbard, pollen grain shape and apertural pattern are invariable (subprolate and 3-colpate), but distinct variation in exine ornamentation is discernible. Four pollen types are recognized on the basis of exine ornamentation (Table 2, Figures 2–5, c, f, i and l). The *S. nivalis* type is characterized by microreticulation and absence of opercula. This type is present in two species, *S. nivalis* and *S. hieraciifolia*, which belong to Section *Micranthes*. The *S. cespitosa* type is characterized by microechinate ornamentation and presence of opercula and is only present in one species, *S. cespitosa*, which belongs to Section *Saxifraga*. The observations of Ferguson and Webb^[7] showed that pollen grains with striate ornamentation are most common in *Saxifraga*. In the present study, the *S.*

oppositifolia type is recognized by striation without scabrae on the muri and is present in Section *Porphyron*. The *S. cernua* type is distinguished by striation with scabrae on the muri. This type is present in four species belonging to three different sections of the genus, viz., *S. cernua*, *S. rivularis* (Section *Mesogyne*), *S. aizoides* (Section *Xanthizoon*), and *S. hirculus* (Section *Ciliatae*). The latter species is distinguished from the former three species by pollen grain shape and *P/E* ratio (prolate vs subprolate, and 1.40 vs about 1.20, respectively). On the basis of pollen grain size, *S. aizoides* (polar axis 20.3 μm and equatorial axis 17.3 μm on average) is distinguished from *S. cernua* and *S. rivularis* (polar axis 26–27 μm and equatorial axis 21–23 μm on average).

Previous studies revealed that the morphological characters of pollen grains of *Saxifraga* (particularly the exine pattern) have taxonomic implications in the genus, not only at the sectional level but in some cases at the species level^[7–8,13–14]. The present study also indicated that exine

ornamentation is a key pollen character in *Saxifraga*, and hierarchical cluster analysis of the pollen characters supports the infrageneric classification of this genus. Our findings suggest that Sections *Mesogyne*, *Xanthizoon*, *Ciliatae*, and *Porphyron* show a close relationship, whereas Section *Micranthes* is distantly related to Section *Saxifraga* and the other sections (Figure 6). This conclusion is in agreement with the results of a phylogenetic analysis of *rbcL* and *matK* chloroplast sequence data in the genus *Saxifraga*, which indicated that *Saxifraga* is polyphyletic and comprises two well-differentiated clades, viz., *Saxifraga sensu stricto* and *Micranthes sensu lato*^[18]. Moreover, it is noteworthy that Section *Micranthes* is distinguished from the other sections by several morphological features, such as a single integument and the presence of longitudinal ribs on the seed testa^[19].

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