JSE Journal of Systematics and Evolution

doi: 10.1111/jse.12149

Research Article

Pollen morphology of *Androsace* (Primulaceae) and its systematic implications

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Received 5 May 2014; Accepted 8 February 2015; Article first published online 21 April 2015

Abstract A close relationship between *Androsace* and related genera (*Douglasia*, *Vitaliana*, and *Pomatosace*) has long been recognized. Recent molecular studies have provided abundant evidence that *Douglasia*, *Vitaliana*, and *Pomatosace* are nested within *Androsace* and together constitute the monophyletic "*Androsace* group". We investigated the pollen morphology of 80 taxa representing all sections of *Androsace* s.s. as traditionally construed, as well as *Douglasia*, *Vitaliana*, and *Pomatosace,* to see whether they are congruent with phylogenetic relationships. We uncovered subtle variation in pollen morphology within the group. The shape of pollen grains ranges from spheroidal to perprolate. Pollen size ranges from 9.37 μm in *Androsace* sect. *Samuelia* to 20.68 μm in *Douglasia*. Exine ornamentation includes microreticulate, microechinate, perforate, and rugulate types. The polar view varies from circular, triangular and planaperturate, to triangular and angulaperturate. Various pollen morphological characters support the monophyly of major clades, including /*Septentrionalis*, /*Pomatosace*, /*Orthocaulon*, and /*Megista*, which were recognized previously based on molecular evidence.

Key words: *Androsace*, *Douglasia*, pollen morphology, *Pomatosace*, SEM, systematics, *Vitaliana*.

The genus *Androsace* L. (Primulaceae) consists of approximately 120 species and is widely distributed in northern temperate areas (Wendelbo, 1961; Hu, 1994; Hu & Kelso, 1996). It is recognized as being distinct from *Primula* L. based on its characteristic corolla structure (a comparatively short corolla tube that has a constricted and annulate throat). *Androsace* and *Primula* are regarded as the two major clades of Primuleae, and several other small allied genera were likely derived from them (Hu & Yang, 1986): the monotypic East Asian *Pomatosace* Maxim., the amphi-Beringian *Douglasia* Lindl., and the monotypic European *Vitaliana* Sesl. are all assumed to be closely related to *Androsace* (Schneeweiss et al., 2004). Some authors treat the latter as separate genera, based on various morphological features (the long corolla tube of *Douglasia*, heterostylous flowers of *Vitaliana*, and the circumscissile capsule of *Pomatosace*; Pax & Knuth, 1905; Constance, 1938; Ferguson, 1972; Yang & Huang, 1989); however, these characters may simply represent autapomorphies.

Handel-Mazzetti (1927) revised Chinese species of *Androsace* and divided the genus into four sections: *Pseudoprimula* Pax (a later homonym and incorrect name), *Aizoidium* Hand.-Mazz., *Chamaejasme* C. Koch, and *Orthocaulon* Hand.-Mazz. Wendelbo (1961) merged *Douglasia* and *Vitaliana* with *Androsace* based on their sharing a constricted and annular corolla tube throat, and assigned species to seven sections: *Samuelia* Schlecht. (corresponding to sect. *Pseudoprimula* of Handel-Mazzetti, 1927), *Aizoidium* Hand.-Mazz., *Chamaejasme* C. Koch, *Orthocaulon* Hand.-Mazz., *Androsace*, *Douglasia* (Lindl.) Wendelbo, and *Vitaliana* (Sesl.) Wendelbo. Hu & Yang (1986) raised subsection *Mirabiles* Hand.-Mazz. of sect. *Samuelia* to the sectional level, based on the possession of hard papery or subleathery leaves in the former. Smith & Lowe (1997) treated cushion-like species distributed in Asia and Europe as sections *Chamaejasme* and *Aretia* (L.) Duby, respectively; both sections can be readily distinguished.

Earlier preliminary pollen morphological studies showed that *Androsace* and three related genera (*Pomatosace*, *Douglasia*, and *Vitaliana*) have a unique pollen type within Primulaceae (the "*Androsace*-type"), in which the pollen grains are prolate and tricolporate (Wendelbo, 1961; Spanowsky, 1962). The results of recent molecular phylogenetic studies based on DNA sequences also support the view that this group of these four genera constitute a major clade of Primulaceae (Källersjö et al., 2000; Mast et al., 2001; Trift et al., 2002; Martins et al., 2003; de Vos et al., 2014). Wang et al. (2004) and Schneeweiss et al. (2004); who respectively focused on the Himalayan and European species of *Androsace*, obtained similar results: (i) the entire *Androsace* group, comprising *Androsace*, *Pomatosace*, *Douglasia* and *Vitaliana*, comprise a clade; (ii) species in the Asian sect. *Chamaejasme* and the European sect. *Aretia* (with a few exceptions) can be divided among two clades corresponding to these sections; and (iii) the annual sect. *Andraspis* (Duby) C. Koch is highly polyphyletic, with multiple phylogenetically independent lineages. A formal systematic treatment of the *Androsace* group has not been attempted due to insufficient taxon sampling in current phylogenetic studies (Wang et al., 2004; Schneeweiss et al., 2004).

Wendelbo (1961) used light microscopy (LM) to describe pollen grains of 48 species in his seven sections, and suggested that pollen size could be used in part to demarcate sectional boundaries. This is the most comprehensive palynological study of *Androsace* to date. However, it provided no additional details, such as exine ornamentation. Nasir (1986) investigated 22 species of *Androsace* occurring in Pakistan and noted that pollen breadth and colpi length were useful at the sectional level of the genus. Punt et al. (1974) investigated only a few species, describing an "*Androsace elongata* type" and an "*Androsace maxima* type"; however, exine ornamentation was unclear in this study.

Here we make new observations of pollen grains in the *Androsace* group using LM and scanning electron microscopy (SEM), to assess possible differences among species and sections (or subgroups). We test previous classifications using these palynological data, and discuss the systematic significance of pollen morphological features for the four genera in this group.

Material and Methods

Taxon sampling

We followed the traditional circumscription of the genus *Androsace* here, with *Douglasia*, *Vitaliana*, and *Pomatosace* treated as separate genera (Pax & Knuth, 1905; Constance, 1938; Ferguson, 1972; Yang & Huang, 1989), but we also considered our results in the context of molecular phylogenetic studies (Schneeweiss et al., 2004;Wang et al., 2004). The sectional delimitation follows the most recent classification of *Androsace* by Smith & Lowe (1997). However, the treatment of Chinese species follows Yang & Huang (1989) and Hu & Kelso (1996) and so sects. *Mirabiles* and *Orthocaulon* are adopted, and *A. delavayi* Franch. and *A. lehmannii* Wall. ex Duby are included in sect. *Chamaejasme* (Table 1). *Androsace barbulata* Ovcz., *A. chamaejasme* Wulfen ex Host subsp. *lehmanniana* (Spreng.) Hultén and *A. capitata* Willd. ex Roem. & Schult. are accepted and treated as members of sect. *Chamaejasme* (Obchinnikov, 1952).

We collected most of the samples from western and southwestern China in fieldtrips in recent years. Voucher specimens are deposited in IBSC (South China Botanical Garden Herbarium; Table 1). Other specimens were sampled from the herbaria of IBSC, PE (Institute of Botany Herbarium, Chinese Academy of Sciences), and US (United States National Herbarium, Smithsonian Institution). In total, we examined 78 taxa, which represent all sections of *Androsace* in addition to representatives of *Pomatosace* and *Douglasia*. For the former, 11 species and 1 subspecies belong to sect. *Samuelia*, considered to be the "most primitive" group of *Androsace* (Hu & Yang, 1986). All the members of the problematic section *Mirabiles* were also included. Pollen grains were gathered from specimens directly and were not fixed. To avoid pollen contamination from other species, most of the pollen grains were gathered from flower buds.

Treatment and observation

Pollen grains were acetolyzed according to the method of Erdtman (1960) and observed with LM and SEM. For LM, acetolyzed pollen grains were mounted in glycerine jelly and sealed with paraffin. For SEM, acetolyzed pollen grains were mounted on stubs, coated with gold, and examined with a JSM-6360LV electron microscope (Jeol, Beijing, China). Pollen grains were randomly chosen to measure the length of the polar axis (*P*), the equatorial diameter (*E*), and the colpus length (*C*). For *P* and *E*, 20 pollen grains were measured at 500× magnification, and 10 grains were measured for *C* at 4000× magnification per sample. Pollen terminology follows Punt et al. (2007).

Results

Pollen grain measurements are given in Table 2, and descriptions of pollen grains for each subgroup of *Androsace* and for related genera are summarized below. The data for *A. maxima* L. (included in *Androsace* sect. *Andraspis*) and *Vitaliana primuliflora* Bertol. are taken from Halbritter & Weber (2005) and Halbritter (2005) respectively.

Androsace

Sect. *Samuelia* (Figs. 1, 2)

Pollen grains radially symmetric, isopolar, tricolporate; 9.37–13.04 (*P*) × 7.25–12.13 (*E*) μm; spheroidal to subprolate (*P*/*E* ratio 1.08–1.33). Colpi long (8.05–10.14 μm), narrow and margins distinct. Endoaperture circular to lalongate, sometimes a bridge across the endoaperture (Figs. 1B, 2N). Exine perforate (Fig. 1) or microreticulate (Fig. 2) with circular lumina; lumina or holes commonly evenly distributed over the whole grain, sometimes obviously reduced at mesocolpium and apocolpium (Figs. 1K, 1L). Equatorial view elliptic to slightly rectangular (e.g., Figs. 1B, 1C, 2D, 2H). Polar view circular with 3 colpi sunken; apertures situated in the area between the obtuse angles (e.g., Figs. 1F, 1I, 2C, 2F).

Sect. *Mirabiles* (Hand.-Mazz.) Yang & Huang (Figs. 3A–3H)

Pollen grains radially symmetric, isopolar, tricolporate; 12.68–17.48 (*P*) × 9.76–11.55 (*E*) μm, subprolate or prolate (*P*/*E* ratio 1.31–1.51). Colpi long (10.00–13.93 μm), narrow and margins distinct. Endoaperture lalongate and a bridge across the endoaperture (Fig. 3A). Exine perforate (Figs. 3D, 3G), and the sparse perforations equally distributed over the whole grain. Equatorial view elliptic (*A. graceae* G. Forrest; Fig. 3H) or rectangular (*A. mirabilis* Franch. and *A. runcinata* Hand.-Mazz.; Figs. 3A, 3B, 3E). Polar view triangular with slightly convex sides and obtuse angles; apertures situated in the middle of the sides (Figs. 3C, 3F).

Sect. *Chamaejasme* (Figs. 4, 5)

Pollen grains radially symmetric, isopolar, tricolporate; 13.50–20.36 (*P*) × 8.34–11.84 (*E*) μm, prolate or perprolate (*P*/*E* ratio 1.38–2.10). Colpi long (10.04–16.26 μm), narrow and margins distinct, sometimes sunken (e.g., Fig. 4B). Endoaperture lalongate (Fig. 5K), sometimes a bridge across the endoaperture (Fig. 5H). Exine rugulate (*A. mollis* Hand.-Mazz.; Figs. 5N, 5O) or microreticulate (e.g., Figs. 4C, 4I, 4L), and dense lumina commonly equally distributed over the whole grain. Equatorial view rectangular or slightly elliptic (e.g., Figs. 4E, 4H, 4N, 5B, 5D). Polar view triangular with slightly convex sides and obtuse angles; apertures situated in the middle of the sides (Figs. 4A, 4D, 4G, 4J, 5A).

Table 1 Sources of materials and voucher information in this palynological study of *Androsace* and related genera

Taxa	Locality	Voucher
Androsace L.		
Sect. *Samuelia* Schlechtd.		
A. alchemilloides Franch.	Yunnan, China	K.-L. Chu 1064 (IBSC)
A. axillaris Franch.	Yunnan, China	Qingzang Exp. 6572 (IBSC)
A. croftii Watt	Xizang, China	Y.-T. Zhang 3416 (IBSC)
A. cuscutiformis Franch.	Chongqing, China	Y. Xu et al. 130041 (IBSC)
A. dissecta Franch.	Yunnan, China	Y. Xu, Xu130064 (IBSC)
A. elatior Pax & Hoffm.	Sichuan, China	Y. Xu et al. 120127 (IBSC)
A. gagnepainiana Hand.-Mazz.	Yunnan, China	T.-T. Yu 19070 (IBSC)
A. geraniifolia Watt	Xizang, China	Qingzang Exp. 6616 (IBSC)
A. henryi Oliv. subsp. *henryi*	Chongqing, China	Y. Xu et al. 130010 (IBSC)
A. henryi Oliv. subsp. *simulans* C. M. Hu & Y. C. Yang	Sichuan, China	X. Li 70398 (IBSC)
A. paxiana R. Knuth	Sichuan, China	Y. Xu et al. 120004 (IBSC)
A. umbellata (Lour.) Merr.	Beijing, China	L.-L. Yang 130043 (IBSC)
Sect. *Mirabiles* (Hand.-Mazz.) Yang & Huang		
A. graceae G. Forrest	Yunnan, China	X. Wu 120131 (IBSC)
A. mirabilis Franch.	Chongqing, China	Y. Xu et al. 130042 (IBSC)
A. runcinata Hand.-Mazz.	Hunan, China	Y. Xu et al. 120108 (IBSC)
Sect. *Chamaejasme* C. Koch		
A. adenocephala Hand.-Mazz.	Xizang, China	G. Hao et al. 120202 (IBSC)
A. akbajtalensis Derganc ex O.Fedtsch.	Deosai, Pakistan	Pakistan Exp. FPH-1212(PE)
A. alaschanica Maxim. var. *alaschanica*	Ningxia, China	Y.-Q. He 2254 (PE)
A. alaschanica Maxim. var. *zadoensis* Y. C. Yang & R. F. Huang	Qinghai, China	Y.-S. Chen 10-1036 (PE)
A. barbulata Ovcz.	Russia	M. Maximova s.n. (PE)
A. bisulca Bureau & Franch. var. *bisulca*	Xizang, China	G. Hao et al. 120262 (IBSC)
A. bisulca Bureau & Franch. var. *aurata* (Petitm.) Y. C. Yang & R. F. Huang	Sichuan, China	Y. Xu, Xu130078 (IBSC)
A. brachystegia Hand.-Mazz.	Sichuan, China	X. Li 73001 (PE)
A. bungeana Schischk.& Bobrov	Russia	S. Thomas et al. 7314 (US)
A. caduca Ovcz.	Russia	P. Ovczinnikov 6688 (PE)
A. capitata Willd. ex Roem. & Schult.	Kamchatskiy region, Penzhinskiy district, Russia	S. Kharkevich 392 (PE)
A. chamaejasme Wulfen ex Host subsp. *chamaejasme*	Hungary	S. Filarszky 388 (PE)
A. chamaejasme Wulfen ex Host subsp. *lehmanniana* (Spreng.) Hultén	N/A	N/A (US# 2312309)
A. cuttingii C. E. C. Fisch.	Xizang, China	C.-Y. Wu 75-391 (IBSC)
A. dasyphylla Bunge	Mongolia	P. Handf 2119 (PE)
A. delavayi Franch.	Yunnan, China	Y. Xu, Xu130113 (IBSC)
A. flavescens Maxim.	Xinjiang, China	Xinjiang Exp. 443 (IBSC)
A. forrestiana Hand.-Mazz.	Sichuan, China	Y. Xu, Xu130087 (IBSC)
A. graminifolia C. E. C. Fisch.	Xizang, China	G. Hao et al. 120214 (IBSC)
A. hookeriana Klatt	Xizang, China	G. Hao et al. 120276 (IBSC)
A. incana Lam.	Xinjiang, China	X.-J. Ge et al., Ge130186 (IBSC)
A. laxa C. M. Hu & Y. C. Yang	Hubei, China	Shennongjia Plant Exp. 10723 (IBSC)
A. lehmanniana Spreng.	Xinjiang, China	X.-J. Ge et al., Ge130112 (IBSC)
A. limprichtii Pax & Hoffm.	Sichuan, China	Y. Xu & X. Wu 120132 (IBSC)
A. longifolia Turcz.	Hebei, China	N/A (PE)
A. mariae Kanitz	Xizang, China	Y. Xu, Xu130125 (IBSC)
A. minor (Hand.-Mazz.) C. M. Hu & Y. C. Yang	Sichuan, China	Y. Xu & X. Wu 120132 (IBSC)
A. mollis Hand.-Mazz.	Xizang, China	Y. Xu, Xu130135 (IBSC)
A. nortonii Ludlow	Xizang, China	Y. Xu, Xu130137 (IBSC)

Continued

Table 1 Continued

Taxa	Locality	Voucher
A. ovczinnikovii Schischk. & Bobrov	Xinjiang, China	A.-R. Li 10407 (IBSC)
A. pomeiensis C. M. Hu & Y. C. Yang	Xizang, China	D.-Y. Hong & J.-S. Ying 0409 (IBSC)
A. rigida Hand.-Mazz.	Yunnan, China	Y. Xu, Xu130052 (IBSC)
A. robusta (R. Knuth) Hand.-Mazz.	Xizang, China	Y.-T. Zhang & K.-Y. Lang 3889 (IBSC)
A. sarmentosa Wall.	Xizang, China	Y.-T. Zhang & K.-Y. Lang 4587 (IBSC)
A. selago Klatt	Xizang, China	Y. Xu 13-0243 (IBSC)
A. spinulifera R. Knuth	Yunnan, China	Y. Xu, Xu130078 (IBSC)
A. squarrosula Maxim.	Xinjiang, China	Xinjiang Exp. 9452 (IBSC)
A. stenophylla (Petitm.) Hand.-Mazz.	Xizang, China	Y. Xu, Xu130145 (IBSC)
A. strigillosa Franch.	Xizang, China	Y. Xu, Xu130184 (IBSC)
A. sublanata Hand.-Mazz.	Yunnan, China	Y. Xu et al., Y2013064 (IBSC)
A. tanggulashanensis Y. C. Yang & R. F. Huang	Xizang, China	K.-Y. Lang 9960 (IBSC)
A. tapete Maxim.	Xizang, China	Y. Xu, Xu130117 (IBSC)
A. villosa L.	Hungary	S. Javorka 390 (PE)
A. wardii W. W. Smith.	Xizang, China	Y. Xu, Xu130116 (IBSC)
A. yargongensis Petitm.	Xizang, China	Y. Xu, Xu130128 (IBSC)
A. zambalensis Hand.-Mazz.	Xizang, China	G. Hao et al. 120286 (IBSC)
Sect. *Aretia* (L.) Duby		
A. alpina Lam.	Zermatt, Swizerland	D.-Y. Hong & Y.-M. Yuan, H96081 (PE)
A. carnea L.	Zermatt, Switzerland	D.-Y. Hong & Y.-M. Yuan, H96103 (PE)
A. obtusifolia All.	Austria	P. Lütken s.n. (IBSC)
A. wulfeniana Sieber ex W. D. J. Koch	Carinthia, Austria	Jahornegg s.n. (PE)
Sect. *Aizoidium* Hand.-Mazz.		
A. bulleyana Forrest	Yunnan, China	Y. Xu, Xu130109 (IBSC)
A. integra Hand.-Mazz.	Sichuan, China	G. Hao et al. 120161 (IBSC)
Sect. *Andraspis* (Duby) C. Koch		
A. filiformis Retz.	Inner Mongolia, China	Z. Wang et al. 603 (PE)
A. lactiflora Pall.	Russia	M. Popov. 325 (IBSC)
A. septentrionalis L. var. *septentrionalis*	Xinjiang, China	X.-J. Ge et al., Ge130092 (IBSC)
A. septentrionalis L. var. *breviscapa* Kryl.	Xinjiang, China	A.-R. Li 0632 (IBSC)
A. raddeana Sommier & Levier	Aragatsotn province, Armenia	G. Fayvush 2540 (IBSC)
Sect. *Orthocaulon* Hand.-Mazz.		
A. erecta Maxim.	Xizang, China	Y. Xu, Xu130176 (IBSC)
Douglasia Lindl.		
D. arctica Hook.	Canada	Welsh 12217 (US)
D. laevigata A. Gray	N/A	N/A (US# 1469856)
D. montana A. Gray	N/A	N/A (US# 58562)
D. nivalis Lindl.	Washington, USA	William s.n. (US)
Pomatosace Maxim.		
P. filicula Maxim.	Qinghai, China	B.-Z. Guo & W.-Y. Wang 11387 (PE)

Species are listed following the classification of Smith & Lowe (1997) with minor modifications. Herbarium abbreviations follow the Index Herbariorum (http://sweetgum.nybg.org/ih/) (Thiers, 2014). N/A, not available.

Sect. *Aretia* (Figs. 6A–6J)
Pollen grains radially symmetric, isopolar, tricolporate; 13.36–16.82 (*P*) × 7.87–11.01 (*E*) μm, prolate (*P/E* ratio 1.49–1.70). Colpi long (9.67–12.09 μm), narrow and margins distinct. Endoaperture circular or slightly lalongate and a bridge across the endoaperture (Figs. 6A, 6D). Exine microreticulate (Figs. 6A, 6B, 6H), and dense lumina commonly equally distributed over the whole grain; or rugulate (Fig. 6E) but perforate at apocolpium and the holes obviously reduced (Fig. 6G). Equatorial view elliptic (Fig. 6C) or rectangular (Fig. 6F). Polar view triangular, sometimes with convex sides; angles obtuse; apertures situated in the middle of the sides (Fig. 6G).

Sect. *Aizoidium* (Figs. 6K–6O)
Pollen grains radially symmetric, isopolar, tricolporate; 16.60–18.68 (*P*) × 10.33–10.34 (*E*) μm, prolate (*P/E* ratio 1.61–1.81). Colpi long (13.48–15.33 μm), narrow and margins distinct, sometimes sunken (Fig. 6L). Endoaperture lalongate. Exine rugulate (Figs. 6K, 6N), but perforate at apocolpium and the holes evenly distributed over this area (Figs. 6M, 6O); sometimes exine is nearly psilate in the middle of mesocolpium (Fig. 6L). Equatorial view rectangular (Fig. 6L). Polar view triangular; angles obtuse; apertures situated in the middle of the sides (Fig. 6O).

Table 2 Pollen morphology characters of *Androsace* and related genera

Taxa	P	E	P/E	C	C/P	W	X	Y	Figure
Androsace									
Sect. *Samuelia*:									
A. alchemilloides	(8.8) 9.88 (10.8)	(6.7) 7.59 (9.3)	(1.12) 1.30 (1.41)	(7.66) 8.37 (9.11)	(0.87) 0.89 (0.91)	1	0	0	2L
A. axillaris	(8.8) 9.69 (10.5)	(7.8) 8.42 (9.4)	(1.04) 1.15 (1.24)	(7.18) 8.05 (8.78)	(0.84) 0.87 (0.90)	1	1	0	1D–1F
A. croftii	(9.2) 10.71 (12.0)	(7.8) 9.00 (10.2)	(1.02) 1.20 (1.50)	(7.10) 8.21 (8.81)	(0.81) 0.86 (0.93)	1	0	1	
A. cuscutiformis	(8.5) 9.37 (10.2)	(7.2) 7.79 (8.2)	(1.09) 1.20 (1.36)	(6.47) 7.57 (8.02)	(0.82) 0.86 (0.89	1	0	1	2A–2C
A. dissecta	(9.4) 9.88 (10.5)	(7.5) 8.12 (8.6)	(1.14) 1.22 (1.35)	(8.17) 8.59 (9.41)	(0.82) 0.87 (0.91)	1	0	0	2D–2F
A. elatior	(8.9) 9.74 (10.2)	(7.3) 8.08 (8.4)	(1.07) 1.21 (1.33)	(7.94) 8.50 (9.27)	(0.88) 0.91 (0.94)	1	1	0	1G–1I
A. gagnepainiana	(12.3) 13.04 (14.4)	(10.8) 12.13 (13.4)	(0.98) 1.08 (1.19)	(9.81) 10.57 (11.45)	(0.79) 0.83 (0.85)	0	0	0	2J, 2K
A. geraniifolia	(9.2) 10.2 (11.5)	(8.3) 9.12 (10.5)	(1.04) 1.12 (1.21)	(8.62) 9.03 (9.77)	(0.86) 0.88 (0.91)	0	0	1	2L
A. henryi subsp. *henryi*	(9.8) 10.5 (11.0)	(8.1) 8.75 (9.4)	(1.1) 1.20 (1.35)	(8.40) 9.29 (10.96)	(0.80) 0.85 (0.90)	1	0	1	2G–2I
A. henryi subsp. *simulans*	(9.5) 9.85 (10.3)	(7.7) 8.15 (8.5)	(1.14) 1.21 (1.29)	(7.44) 8.16 (9.05)	(0.79) 0.84 (0.90)	1	1	0	1A–1C
A. paxiana	(11.0) 11.79 (12.8)	(9.8) 10.65 (11.7)	(1.01) 1.11 (1.23)	(10.06) 10.14 (10.22)	(0.85) 0.88 (0.90)	0	0	0	2N, 2O
A. umbellata	(8.9) 9.63 (10.1)	(6.8) 7.25 (7.9)	(1.18) 1.33 (1.41)	(8.39) 8.53 (8.90)	(0.90) 0.92 (0.94)	1	1	1	1J–1L
Sect. *Mirabiles*:									
A. graceae	(11.2) 12.68 (14.7)	(8.4) 9.76 (11.6)	(1.12) 1.31 (1.54)	(8.61) 10.00 (12.31)	(0.74) 0.81 (0.91)	1	1	0	3G, 3H
A. mirabilis	(13.6) 14.57 (15.3)	(9.9) 10.74 (11.5)	(1.27) 1.36 (1.48)	(9.99) 10.98 (12.34)	(0.75) 0.80 (0.86)	2	1	0	3A–3D
A. runcinata	(14.9) 17.48 (18.8)	(10.8) 11.55 (12.3)	(1.36) 1.51 (1.62)	(11.79) 13.93 (15.06)	(0.78) 0.82 (0.84)	2	1	0	3E, 3F
Sect. *Chamaejasme*:									
A. adenocephala	(13.2) 15.84 (17.7)	(8.4) 9.34 (10.1)	(1.31) 1.70 (2.01)	(11.19) 12.07 (13.24)	(0.75) 0.80 (0.86)	2	0	0	
A. akbajtalensis	(12.8) 13.90 (15.3)	(8.5) 9.15 (9.9)	(1.32) 1.52 (1.66)	(9.18) 10.30 (11.00)	(0.72) 0.77 (0.81)	2	0	1	
A. alaschanica var. *alaschanica*	(14.2) 14.85 (15.9)	(7.7) 8.55 (9.4)	(1.61) 1.74 (1.89)	(11.30) 12.09 (13.00)	(0.74) 0.82 (0.86)	2	0	0	4F
A. alaschanica var. *zadoensis*	(13.2) 14.96 (17.2)	(7.9) 9.06 (10.4)	(1.52) 1.65 (1.94)	(10.20) 11.34 (12.80)	(0.70) 0.81 (0.96)	2	0	1	
A. barbulata	(12.3) 13.50 (14.5)	(8.2) 8.63 (9.3)	(1.43) 1.57 (1.68)	(9.66) 10.26 (10.90)	(0.71) 0.76 (0.82)	2	0	1	5A–5C
A. bisulca var. *bisulca*	(13.5) 15.32 (16.7)	(8.7) 9.50 (10.7)	(1.40) 1.62 (1.84)	(10.16) 11.78 (13.46)	(0.70) 0.78 (0.84)	2	0	1	4N
A. bisulca var. *aurata*	(15.6) 16.60 (19.3)	(9.1) 9.94 (10.8)	(1.47) 1.67 (1.97)	(10.98) 12.75 (14.65)	(0.72) 0.80 (0.86)	2	0	1	5D, 5E
A. brachystegia	(15.0) 16.05 (17.7)	(8.8) 9.36 (9.8)	(1.54) 1.72 (1.88)	(10.98) 12.17 (14.6)	(0.73) 0.79 (0.84)	2	0	1	4K
A. bungeana	(13.8) 14.96 (16.4)	(8.3) 9.07 (10.1)	(1.50) 1.65 (1.78)	(11.70) 12.45 (13.20)	(0.80) 0.85 (0.92)	2	0	1	
A. caduca	(12.9) 14.74 (16.2)	(8.9) 9.44 (10.4)	(1.24) 1.56 (1.74)	(10.00) 11.49 (12.48)	(0.72) 0.82 (0.85)	2	0	1	4M
A. capitata	(13.8) 15.54 (16.7)	(8.8) 9.54 (10.4)	(1.44) 1.63 (1.78)	(10.93) 12.20 (12.9)	(0.78) 0.81 (0.85)	2	0	1	5H, 5I
A. chamaejasme subsp. *chamaejasme*	(13.6) 14.58 (15.5)	(8.0) 8.78 (9.9)	(1.55) 1.66 (1.94)	(10.10) 11.36 (12.68)	(0.75) 0.80 (0.87)	2	0	1	
A. chamaejasme subsp. *lehmanniana*	(14.3) 15.62 (18.2)	(9.5) 10.25 (10.8)	(1.42) 1.52 (1.69)	(10.27) 11.92 (13.45)	(0.76) 0.84 (0.88)	2	0	1	5K
A. cuttingii	(13.7) 14.34 (15.5)	(8.1) 8.67 (9.5)	(1.54) 1.66 (1.82)	(10.30) 11.23 (12.14)	(0.74) 0.81 (0.86)	2	0	1	
A. dasyphylla	(13.0) 14.13 (15.1)	(8.9) 9.14 (9.9)	(1.38) 1.55 (1.66)	(11.30) 11.72 (12.60)	(0.79) 0.82 (0.86)	2	0	1	
A. delavayi	(15.1) 16.15 (17)	(9.5) 10.02 (10.5)	(1.48) 1.61 (1.79)	(10.03) 11.35 (12.4)	(0.71) 0.75 (0.78)	2	0	0	4O
A. flavescens	(14.5) 15.21 (16.6)	(8.2) 8.99 (9.9)	(1.53) 1.70 (1.90)	(10.60) 11.44 (12.02)	(0.72) 0.80 (0.85)	2	0	1	
A. forrestiana	(16.2) 17.40 (19)	(9.2) 9.60 (10.1)	(1.66) 1.82 (1.97)	(12.00) 12.79 (13.76)	(0.71) 0.77 (0.85)	2	0	1	
A. graminiflora	(12.6) 14.82 (16.6)	(8.3) 9.30 (10.7)	(1.31) 1.60 (1.76)	(10.24) 11.75 (12.5)	(0.78) 0.81 (0.86)	2	0	1	
A. hookeriana	(14.9) 16.46 (17.9)	(7.4) 8.34 (9.6)	(1.57) 1.98 (2.16)	(11.40) 12.46 (14.00)	(0.69) 0.76 (0.84)	2	0	1	

Continued

Table 2 Continued

Taxa	*P*	*E*	*P/E*	*C*	*C/P*	W	X	Y	Figure
A. incana	(13.6) 14.68 (16)	(9.9) 10.67 (11.6)	(1.25) 1.38 (1.49)	(10.25) 11.60 (12.50)	(0.76) 0.81 (0.85)	2	0	1	5L
A. laxa	(15.3) 18.07 (20)	(8.9) 10.82 (13.3)	(1.42) 1.68 (1.92)	(10.95) 11.78 (12.55)	(0.68) 0.74 (0.81)	2	0	1	
A. lehmanniana	(13.2) 15.00 (17.3)	(8.8) 9.42 (10.1)	(1.38) 1.59 (1.86)	(9.96) 11.11 (11.79)	(0.72) 0.78 (0.83)	2	0	0	
A. limprichtii	(14.6) 15.68 (17.1)	(8.3) 9.08 (9.8)	(1.60) 1.73 (1.93)	(9.31) 11.25 (12.52)	(0.64) 0.75 (0.81)	2	0	0	
A. longifolia	(15.6) 17.23 (19.1)	(9.2) 9.91 (11.0)	(1.50) 1.74 (1.91)	(12.40) 13.21 (14.40)	(0.73) 0.78 (0.83)	2	0	0	
A. mariae	(14.6) 16.61 (17.9)	(9.4) 10.15 (11.6)	(1.36) 1.64 (1.83)	(9.30) 11.33 (12.40)	(0.61) 0.71 (0.80)	2	0	1	4D
A. minor	(13.7) 15.47 (18.3)	(8.2) 8.63 (9.3)	(1.51) 1.80 (2.13)	(10.05) 11.33 (12.50)	(0.71) 0.78 (0.85)	2	0	1	
A. mollis	(15.8) 18.02 (20.2)	(9.8) 11.77 (13.3)	(1.41) 1.54 (1.71)	(10.06) 12.50 (15.31)	(0.66) 0.76 (0.89)	2	2	1	5N, 5O
A. nortonii	(14.5) 16.18 (17.5)	(8.7) 9.39 (10.4)	(1.48) 1.72 (1.85)	(10.18) 11.88 (13.9)	(0.69) 0.77 (0.85)	2	0	0	5J
A. ovczinnikovii	(12.6) 14.18 (15.3)	(8.4) 9.35 (11.3)	(1.33) 1.52 (1.71)	(9.14) 10.20 (11.42)	(0.75) 0.79 (0.82)	2	0	1	5I
A. pomeiensis	(13.4) 14.89 (17.2)	(8.7) 9.47 (10.0)	(1.41) 1.57 (1.77)	(10.23) 10.87 (11.80)	(0.72) 0.77 (0.81)	2	0	1	4E
A. rigida	(18.5) 20.36 (21.6)	(8.5) 9.75 (11.3)	(1.81) 2.10 (2.40)	(13.88) 15.03 (16.09)	(0.72) 0.76 (0.80)	3	0	1	4A, 4B
A. robusta	(12.2) 13.84 (16.1)	(8.6) 9.83 (11.0)	(1.19) 1.41 (1.58)	(9.49) 10.04 (10.52)	(0.78) 0.81 (0.82)	2	0	0	
A. sarmentosa	(15.1) 16.78 (18.5)	(9.9) 10.85 (12.0)	(1.39) 1.55 (1.75)	(11.56) 13.20 (15.78)	(0.74) 0.79 (0.86)	2	0	0	5H
A. selago	(15.1) 16.43 (18.5)	(9.7) 11.84 (13.1)	(1.20) 1.40 (1.85)	(12.60) 13.19 (13.77)	(0.75) 0.81 (0.88)	2	0	0	
A. spinulifera	(15.2) 16.74 (20.6)	(8.6) 9.22 (10.0)	(1.67) 1.82 (2.10)	(10.5) 12.33 (14.2)	(0.67) 0.75 (0.82)	2	0	1	5M
A. squarrosula	(14.4) 15.87 (17.4)	(8.6) 9.65 (11.0)	(1.45) 1.65 (1.96)	(12.24) 12.99 (14.34)	(0.80) 0.84 (0.87)	2	0	1	
A. stenophylla	(13.5) 14.36 (15.8)	(8.4) 8.96 (9.5)	(1.48) 1.60 (1.78)	(9.64) 10.65 (11.74)	(0.67) 0.74 (0.83)	2	0	1	5G
A. strigillosa	(15.3) 16.85 (18.6)	(8.8) 9.97 (11.0)	(1.54) 1.69 (1.86)	(9.71) 12.52 (14.17)	(0.71) 0.81 (0.86)	2	0	1	
A. sublanata	(18.1) 19.32 (20.5)	(9.5) 10.18 (11.2)	(1.83) 1.90 (2.05)	(13.40) 15.17 (16.12)	(0.71) 0.80 (0.84)	2	0	1	
A. tanggulashanensis	(17.4) 19.42 (21.8)	(8.6) 10.22 (11.3)	(1.69) 1.91 (2.15)	(13.9) 15.77 (17.00)	(0.77) 0.82 (0.88)	2	0	1	
A. tapete	(13.9) 15.11 (16.3)	(8.4) 8.78 (9.6)	(1.56) 1.72 (1.90)	(11.27) 12.07 (12.8)	(0.80) 0.86 (0.90)	2	0	1	4C
A. villosa	(13.7) 14.82 (16.0)	(8.7) 9.37 (10.8)	(1.38) 1.59 (1.80)	(10.50) 11.06 (12.20)	(0.73) 0.79 (0.84)	2	0	1	5F
A. wardii	(15.4) 16.95 (18.8)	(8.6) 9.25 (9.7)	(1.62) 1.84 (2.12)	(11.80) 12.59 (13.20)	(0.72) 0.78 (0.80)	2	0	1	4G
A. yargongensis	(18.1) 19.95 (20.8)	(10.6) 11.50 (12.4)	(1.56) 1.74 (1.90)	(14.29) 16.26 (17.8)	(0.75) 0.83 (0.89)	2	0	1	4J, 4L
A. zambalensis	(19.0) 19.88 (20.9)	(10.1) 10.86 (11.9)	(1.67) 1.83 (2.02)	(12.15) 15.29 (16.92)	(0.66) 0.78 (0.87)	2	0	1	
Sect. *Aretia*:									
A. alpina	(12.4) 13.36 (13.9)	(7.1) 7.87 (8.4)	(1.52) 1.70 (1.84)	(8.45) 9.67 (10.40)	(0.69) 0.75 (0.80)	2	0	0	6A–6C
A. carnea	(15.8) 16.82 (18.2)	(9.7) 10.54 (11.7)	(1.47) 1.60 (1.75)	(11.03) 12.20 (13.20)	(0.67) 0.73 (0.77)	2	2	0	6D–6G
A. obtusifolia	(13.3) 14.96 (16.9)	(8.0) 9.06 (11.2)	(1.36) 1.66 (1.97)	(11.20) 11.66 (12.70)	(0.80) 0.82 (0.89)	2	0	0	6H, 6I
A. wulfeniana	(14.0) 16.39 (17.9)	(9.8) 11.01 (12.2)	(1.29) 1.49 (1.69)	(11.2) 12.09 (13.21)	(0.67) 0.72 (0.76)	2	2	0	6J
Sect. *Aizoidium*:									
A. bulleyana	(17.3) 18.68 (21.4)	(9.8) 10.34 (11.0)	(1.61) 1.81 (2.04)	(14.35) 15.33 (16.7)	(0.78) 0.84 (0.89)	2	2	1	6K–6M
A. integra	(15.7) 16.60 (17.7)	(9.7) 10.33 (10.8)	(1.52) 1.61 (1.70)	(13.10) 13.48 (14.29)	(0.78) 0.82 (0.89)	2	2	1	6N, 6O
Sect. *Andraspis*:									
A. filiformis	(12.6) 14.04 (15.9)	(10.4) 11.42 (12.6)	(1.08) 1.23 (1.33)	(11.50) 12.80 (13.30)	(0.90) 0.94 (0.98)	1	0	1	7J–7L
A. lactiflora	(10.9) 12.37 (13.5)	(6.8) 7.71 (8.5)	(1.45) 1.61 (1.87)	(8.70) 9.27 (10.00)	(0.78) 0.82 (0.84)	2	2	1	
A. septentrionalis var. *septentrionalis*	(11.2) 12.25 (13.2)	(6.4) 7.72 (8.4)	(1.44) 1.59 (1.78)	(9.67) 10.27 (10.99)	(0.79) 0.84 (0.91)	2	2	0	7A–7D
A. septentrionalis var. *breviscapa*	(11.9) 13.26 (14.3)	(7.5) 8.13 (8.8)	(1.48) 1.63 (1.76)	(8.98) 9.91 (10.90)	(0.74) 0.80 (0.84)	2	2	0	7E, 7F

Continued

Table 2 Continued

Taxa	P	E	P/E	C	C/P	W	X	Y	Figure
A. raddeana	(12.0) 13.19 (14.4)	(8.0) 8.78 (9.4)	(1.34) 1.51 (1.76)	(8.63) 10.14 (11.40)	(0.71) 0.74 (0.79)	2	2	0	7G–7I
Sect. *Orthocaulon*									
A. erecta	(11.0) 12.17 (12.9)	(8.5) 8.97 (9.8)	(1.18) 1.36 (1.52)	(9.11) 9.82 (10.49)	(0.77) 0.85 (0.89)	2	0	0	3I–3L
Douglasia									
D. arctica	(19.1) 20.31 (21.5)	(12.2) 13.63 (14.4)	(1.38) 1.49 (1.64)	(13.48) 14.76 (15.93)	(0.70) 0.75 (0.82)	2	2	0	8A, 8B
D. laevigata	(19.5) 20.38 (21.2)	(11.9) 12.54 (13.4)	(1.51) 1.63 (1.75)	(12.29) 14.80 (16.40)	(0.69) 0.75 (0.80)	2	2	0	8C–8F
D. montana	(16.3) 18.63 (19.8)	(9.6) 11.31 (12.2)	(1.57) 1.65 (1.78)	(14.00) 14.78 (15.80)	(0.80) 0.82 (0.88)	2	1	0	8G–8I
D. nivalis	(19.5) 20.68 (21.5)	(12.1) 13.04 (14.4)	(1.38) 1.59 (1.76)	(10.86) 12.13 (14.15)	(0.55) 0.62 (0.71)	2	2	0	8J–8L
Pomatosace									
P. filicula	(13.4) 14.67 (16.1)	(10.5) 11.17 (12.1)	(1.13) 1.31 (1.46)	(11.80) 12.15 (12.50)	(0.78) 0.83 (0.88)	1	0	1	8M–8O

C and *C/P*, colpus length (μm) and colpus length in proportion to polar axis; *P*, *E*, and *P/E*: length of polar axis (μm), equatorial diameter (μm), and ratio P/E (measure for pollen grain shape); (minimum), mean, (maximum) values. W, Pollen shape: 0, spheroidal; 1, subprolate; 2, prolate; 3, perprolate. X, Exine ornamentation: 0, microreticulate; 1, perforate; 2, rugulate; 3, microechinate. Y, bridge: 0, bridge across the endoaperture; 1, no bridge.

Sect. *Andraspis* (Fig. 7)

Pollen grains radially symmetric, isopolar, tricolporate; 12.25–14.04 (*P*) × 7.71–11.42 (*E*) μm, subprolate or prolate (*P/E* ratio 1.23–1.63). Colpi long (9.27–12.80 μm), narrow and margins distinct, sometimes sunken (*A. filiformis* Retz.; Fig. 7L). Endoaperture lalongate and sometimes a bridge across the endoaperture (Figs. 7B, 7F, 7I). Exine microreticulate and dense lumina commonly equally distributed over the whole grain (Fig. 7J); rugulate but perforate at apocolpium and dense holes evenly distributed over this area (Figs. 7A, 7D, 7E, 7G); or microechinate (*A. maxima*). Equatorial view elliptic (e.g., Figs. 7C, 7K) or suborbicular (*A. maxima*). Polar view circular (*A. filiformis*) or triangular, sometimes with convex sides; angles obtuse; apertures situated in the middle of the sides (Fig. 7I) or at the angles (*A. maxima*).

Sect. *Orthocaulon* (Figs. 3I–3L)

Pollen grains radially symmetric, isopolar, tricolporate; (11.0) 12.17 (12.9) (*P*) × (8.5) 8.97 (9.8) (*E*) μm, prolate (*P/E* ratio 1.18–1.52). Colpi long (9.11–10.49 μm), narrow and margins distinct. Endoaperture lalongate and a bridge across the endoaperture (Fig. 3K). Exine microreticulate (Fig. 3J), and dense lumina commonly equally distributed over the whole grain. Equatorial view rectangular (Figs. 3K, 3L). Polar view circular with three sunken colpi; apertures situated in the area between the obtuse angles (Fig. 3I).

Douglasia (Figs. 8A–8L)

Pollen grains radially symmetric, isopolar, tricolporate; 18.63–20.68 (*P*) × 11.31–13.63 (*E*) μm, prolate (*P/E* ratio 1.49–1.65). Colpi long (12.13–14.80 μm), narrow and margins distinct, sometimes sunken (*Douglasia nivalis* Lindl.; Fig. 8K). Endoaperture circular and a bridge across the endoaperture (Fig. 8L). Exine rugulate with wider muri but perforate at apocolpium with sparse holes (Figs. 8A, 8D, 8F, 8J); or perforate and dense lumina commonly equally distributed over the whole grain (Fig. 8G). Equatorial view elliptic (Figs. 8B, 8E, 8H, 8K). Polar view triangular; angles obtuse; apertures situated in the middle of the sides (Fig. 8C).

Pomatosace (Figs. 8M–8O)

Pollen grains radially symmetric, isopolar, tricolporate; (13.4) 14.67 (16.1) (*P*) × (10.5) 11.17 (12.1) (*E*) μm, subprolate (*P/E* ratio 1.13–1.46). Colpi long (11.80–12.50 μm), narrow and margins distinct (Fig. 8N). Endoaperture lalongate. Exine microreticulate with wider muri and irregular lumina (Fig. 8M); dense lumina evenly distributed over the whole grain. Equatorial view rectangular (Fig. 8N). Polar view circular with three sunken colpi; apertures situated in the area between the obtuse angles (Fig. 8O).

Discussion

Variations of pollen morphological characters of the *Androsace* group

Characters of the pollen grains have abundant subtle variations. The pollen shape varies from spheroidal to perprolate, and the exine ornamentation has four states (microreticulate, perforate, rugulate, and microechinate), but these states can be shared or overlapping among subgroups

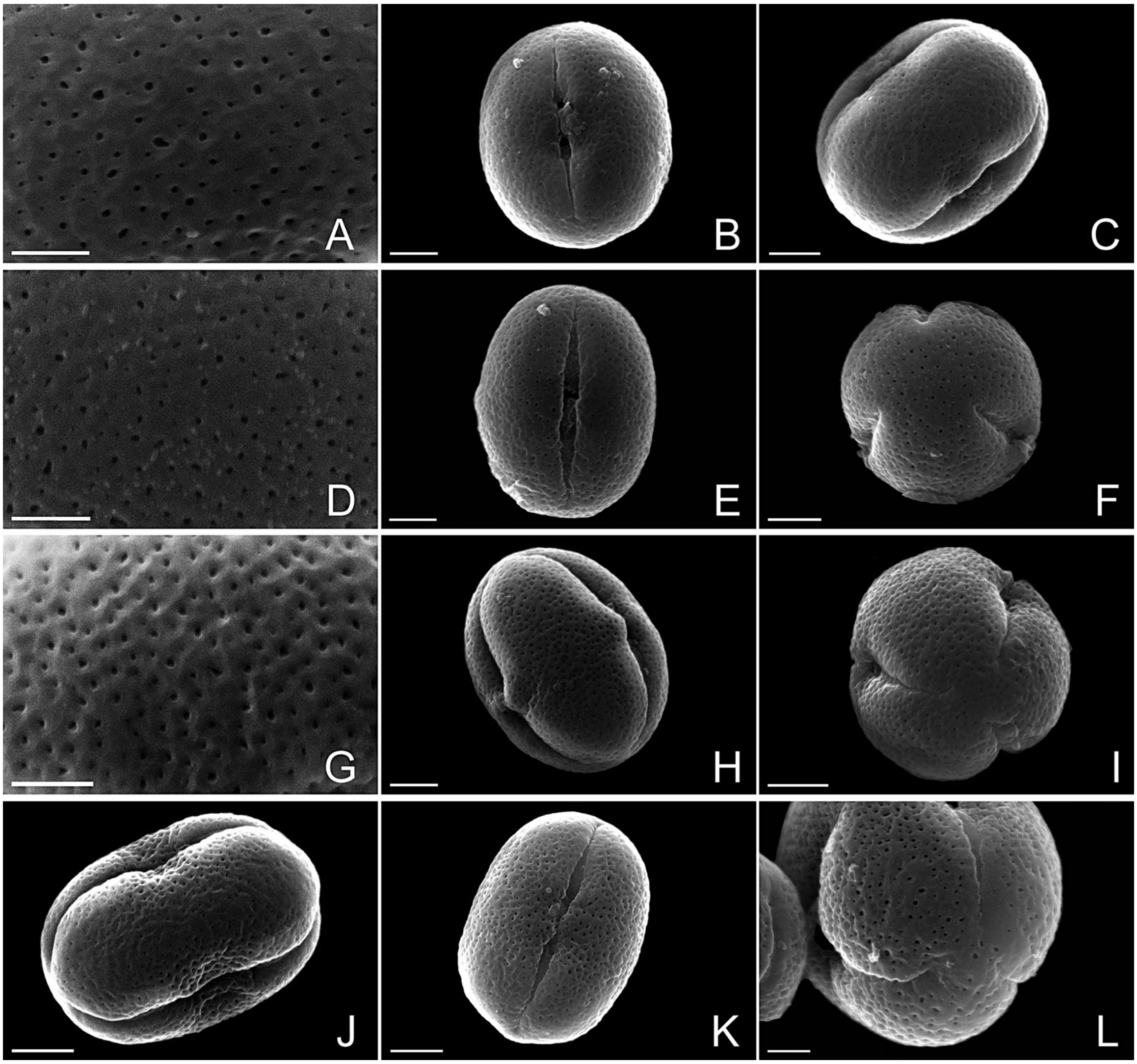

Fig. 1. Scanning electron micrographs of pollen grains from *Androsace* sect. *Samuelia*, showing the perforate condition. **A–C,** *Androsace henryi* subsp. *simulans*. **A,** Detail of exine ornamentation at mesocolpium. **B,** Equatorial view showing the bridge. **C,** Equatorial view. **D–F,** *A. axillaris*. **D,** Detail of exine ornamentation at mesocolpium. **E,** Equatorial view. **F,** Polar view. **G–I,** *A. elatior*. **G,** Detail of exine ornamentation at mesocolpium. **H,** Equatorial view. **I,** Polar view. **J–L,** *A. umbellata*. **J,** Equatorial view. **K,** Equatorial view. **L,** Polar view. Scale bar = 2 μm (B, C, E, F, H–L) or 1 μm (A, D, G).

(sections) of *Androsace* and related genera (Table 2; Fig. 9). Pollen size of examined pollen grains is very small (<10 μm) to small (10–25 μm), ranging from 9.37 μm (*A. cuscutiformis* Franch.; sect. *Samuelia*) to 20.68 μm (*Douglasia laevigata* A. Gray). The variations of colpi length are continuous in different sections, and the *C/P* ratio is homogeneous across different *Androsace* sections. Therefore, our results do not support the view of Wendelbo (1961) and Nasir (1986) that pollen size and colpi length are significant in distinguishing sections of *Androsace*.

Except for the overlapping and continuous variations, we observed some intermediate characters between microreticulate and rugulate in exine ornamentation. For example, *A. incana* Lam. has a partly rugulate exine ornamentation (Fig. 5L); *A. ovczinnikovii* Schischk. & Bobrov has slightly ridged muri (Fig. 5I); and the muri of *A. villosa* L. and *A. obtusifolia* All. are shorter than 1 μm (Figs. 5F, 6H). All these intermediate characters are treated as microreticulate.

At the species level, *A. maxima* has the most unique pollen morphotype of all the examined pollen grains, characterized by a microechinate exine ornamentation and angulaperturate polar view. This confirms the view of Punt et al. (1974), who stated that the *Androsace maxima* type can be clearly defined. However, the *Androsace elongata* type of Punt et al. (1974), which is found in *A. carnea* L. (of sect. *Aretia*; Figs. 6D–6G), *A. elongata* L. (of sect. *Andraspis*), *A. lactea* L. (of sect. *Aretia*), *A. septentrionalis* L. (of sect. *Andraspis*; Figs. 7A–7D) and *A. villosa* (of sect. *Chamaejasme*; Fig. 5F), is more problematic. Based on our increased taxon sampling and more detailed observations of exine ornamentation, we find that all the characters that comprise the *Androsace elongata* type can be found in other subgroups, including its microreticulate or

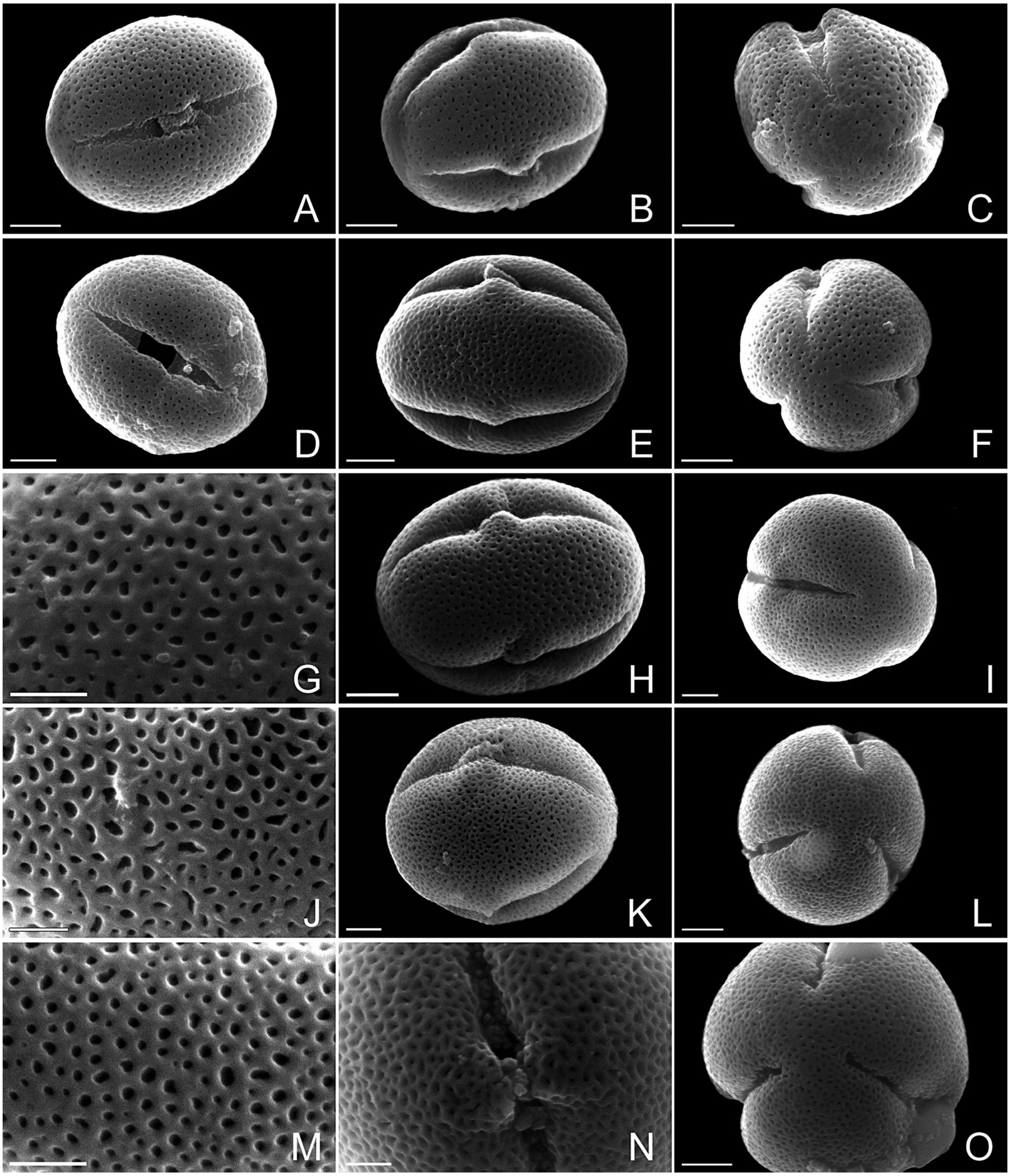

Fig. 2. Scanning electron micrographs of pollen grains from *Androsace* sect. *Samuelia*, showing the microreticulate condition. **A–C,** *Androsace cuscutiformis*. **A,** Equatorial view. **B,** Oblique equatorial view. **C,** Polar view. **D–F,** *A. dissecta*. **D,** Equatorial view. **E,** Equatorial view. **F,** Polar view. **G–I,** *A. henryi*. **G,** Detail of exine ornamentation at mesocolpium. **H,** Equatorial view. **I,** Oblique polar view. **J, K,** *A. gagnepainiana*. **J,** Detail of exine ornamentation at mesocolpium. **K,** Equatorial view. **L,** *A. geraniifolia*, polar view. **M,** *A. alchemilloides*, detail of exine ornamentation at mesocolpium. **N, O,** *A. paxiana*. **N,** Detail of bridge. **O,** Polar view. Scale bar = 2 μm (A–F, H, I, K, L, O) or 1 μm (G, J, M, N).

perforate exine, perprolate shape, rectangular or elliptic equatorial view, and triangular polar view.

Systematic implications of pollen morphology

The pollen morphology of *Androsace* and related genera is unique within Primulaceae, and we confirm that the group is defined by tricolporate pollen grains with prominent aperture (Wendelbo, 1961; Spanowsky, 1962). The monophyly of the *Androsace* group has also been confirmed by evidence from molecular phylogenetic analyses (Källersjö et al., 2000; Mast et al., 2001; Trift et al., 2002; Martins et al., 2003; Schneeweiss et al., 2004; Wang et al., 2004; Boucher et al., 2012; de Vos

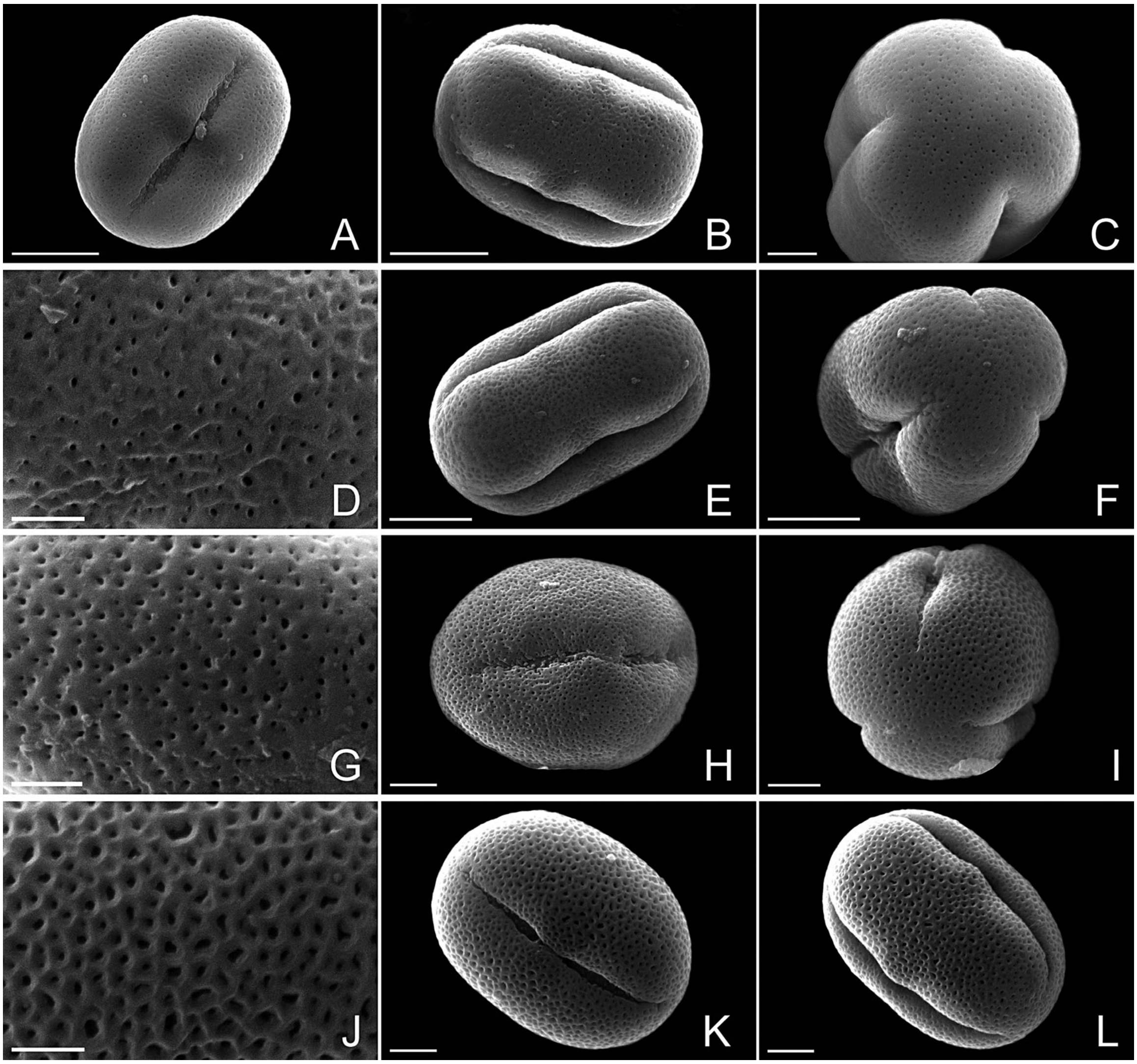

Fig. 3. Scanning electron micrographs of pollen grains from *Androsace* sect. *Mirabiles* and sect. *Orthocaulon*. **A–D,** *Androsace mirabilis*. **A,** Equatorial view. **B,** Equatorial view. **C,** Polar view. **D,** Detail of exine ornamentation at mesocolpium. **E, F,** *A. runcinata*. **E,** Equatorial view. **F,** Polar view. **G, H,** *A. graceae*. **G,** Detail of exine ornamentation at mesocolpium. **H,** Equatorial view. **I–L,** *A. erecta*. **I,** Polar view. **J,** Detail of exine ornamentation at mesocolpium. **K,** Equatorial view. **L,** Equatorial view. Scale bar = 5 μm (A, B, E, F), 2 μm (C, H, I, K, L) or 1 μm (D, G, J).

et al., 2014). A formal taxonomic treatment of the *Androsace* group has not yet been proposed, because of insufficient sampling (especially of the Chinese species from the species-rich sections *Samuelia* and *Chamaejasme*). However, a clade-based classification has been suggested by Schneeweiss et al. (2004). Unique characters to distinguish subgroups of the *Androsace* group are lacking, but combinations of pollen morphological characters may partly reflect phylogenetic relationships. We therefore mapped the main pollen characters (exine ornamentation, shape, and polar view) on the most recent phylogenetic tree of de Vos et al. (2014) (Fig. 9), and discuss the systematic implications here.

Although the molecular analysis sampled only one species (*A. cuscutiformis*) from sect. *Samuelia*, Schneeweiss et al. (2004) pointed out that /*Pseudoprimula* (corresponding to sect. *Samuelia* of Yang & Huang, 1989) is the only clade that corresponds to a traditionally recognized section. Subsequent studies by Boucher et al. (2012) and de Vos et al. (2014) that included a few additional *Samuelia* species (*A. axillaris* Franch. and *A. elatior* Pax & Hoffm.) also supported this view. In our palynological investigation, species of sect. *Samuelia* can be separated from other subgroups by their characteristic small size, perforate or microreticulate exine, and a circular polar view (Figs. 1, 2). Based on its unique leaf morphology (broad blades with long petioles), sect. *Samuelia* has usually been treated as an independent section of *Androsace* (Pax & Knuth, 1905; Wendelbo, 1961; Hu & Yang, 1986; Smith & Lowe, 1997) and has even been raised to the genus level (Schwarz, 1963).

Section *Mirabiles* (Figs. 3A–3H), which has only three species, is distinct from sect. *Samuelia* in having hard papery or subleathery leaves. Although the leaf character in the former section is invariant, it has two pollen morphology patterns.

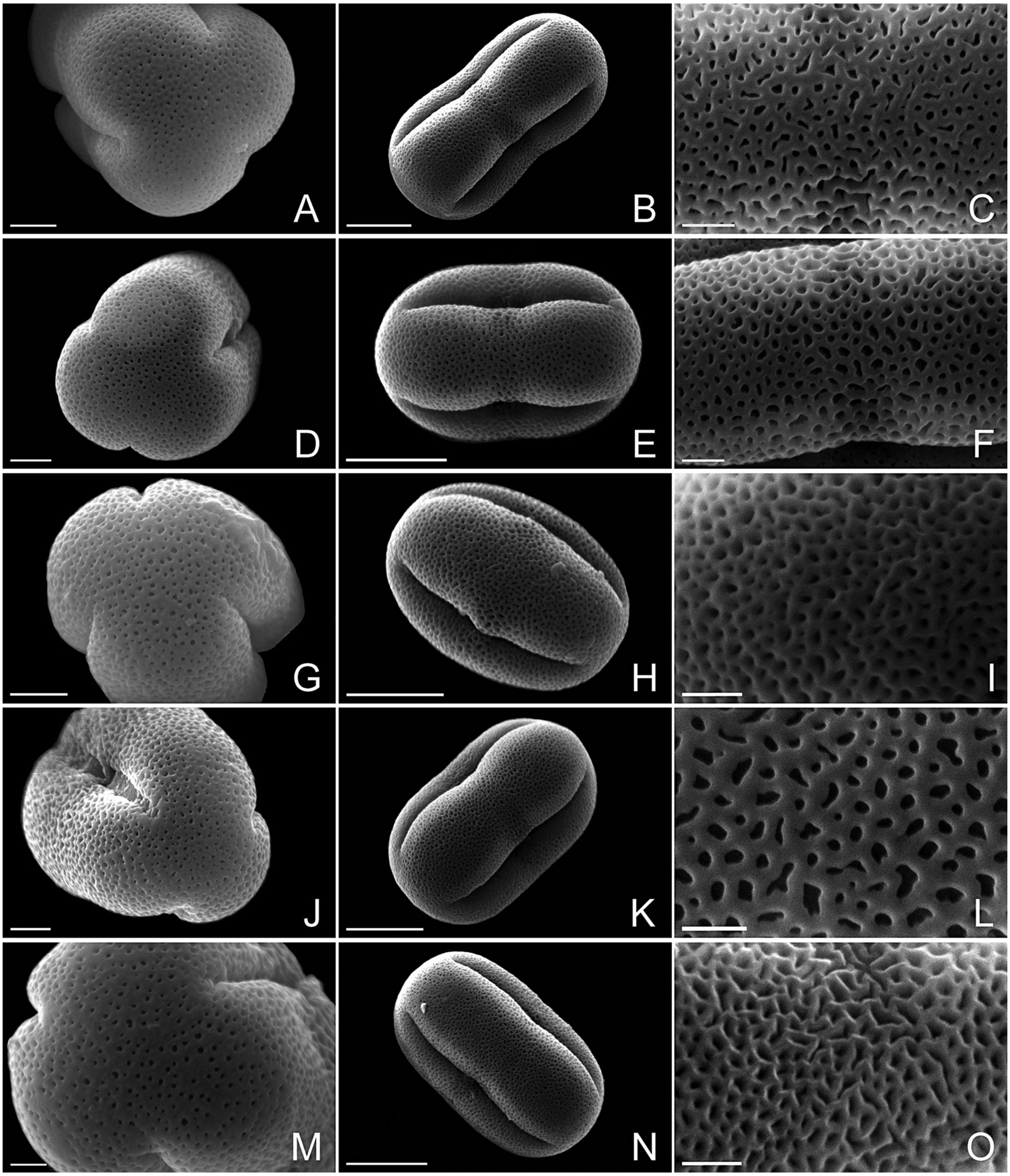

Fig. 4. Scanning electron micrographs of pollen grains from *Androsace* sect. *Chamaejasme*. **A, B,** *Androsace rigida*. **A,** Polar view. **B,** Equatorial view. **C,** *A. tapete*, detail of exine ornamentation at mesocolpium. **D,** *A. mariae*, polar view. **E,** *A. pomeiensis*, equatorial view. **F,** *A. alaschanica*, detail of exine ornamentation at mesocolpium. **G,** *A. wardii*, polar view. **H, I,** *A. capitata*. **H,** Equatorial view. **I,** Detail of exine ornamentation at mesocolpium. **J, L,** *A. yargongensis*. **J,** Oblique polar view. **L,** Detail of exine ornamentation at mesocolpium. **K,** *A. brachystegia*, equatorial view. **M,** *A. caduca*, detail of exine ornamentation at apocolpium. **N,** *A. bisulca*, equatorial view. **O,** *A. delavayi*, detail of exine ornamentation at mesocolpium. Scale bar = 5 μm (B, E, H, K, N), 2 μm (A, D, G, J), or 1 μm (C, F, I, L, M, O).

Pollen grains of *A. graceae* are similar to sect. *Samuelia* in having a subprolate shape and perforate exine (Figs. 3G–3H), whereas *A. mirabilis* and *A. runcinata* are unique in having a prolate shape, perforate exine, and triangular polar view (Figs. 3A–3F). To date, however, no molecular studies have examined the systematics of this section.

The East Asian short-lived species, *A. erecta* Maxim., which was not fully included in the investigation of Schneeweiss

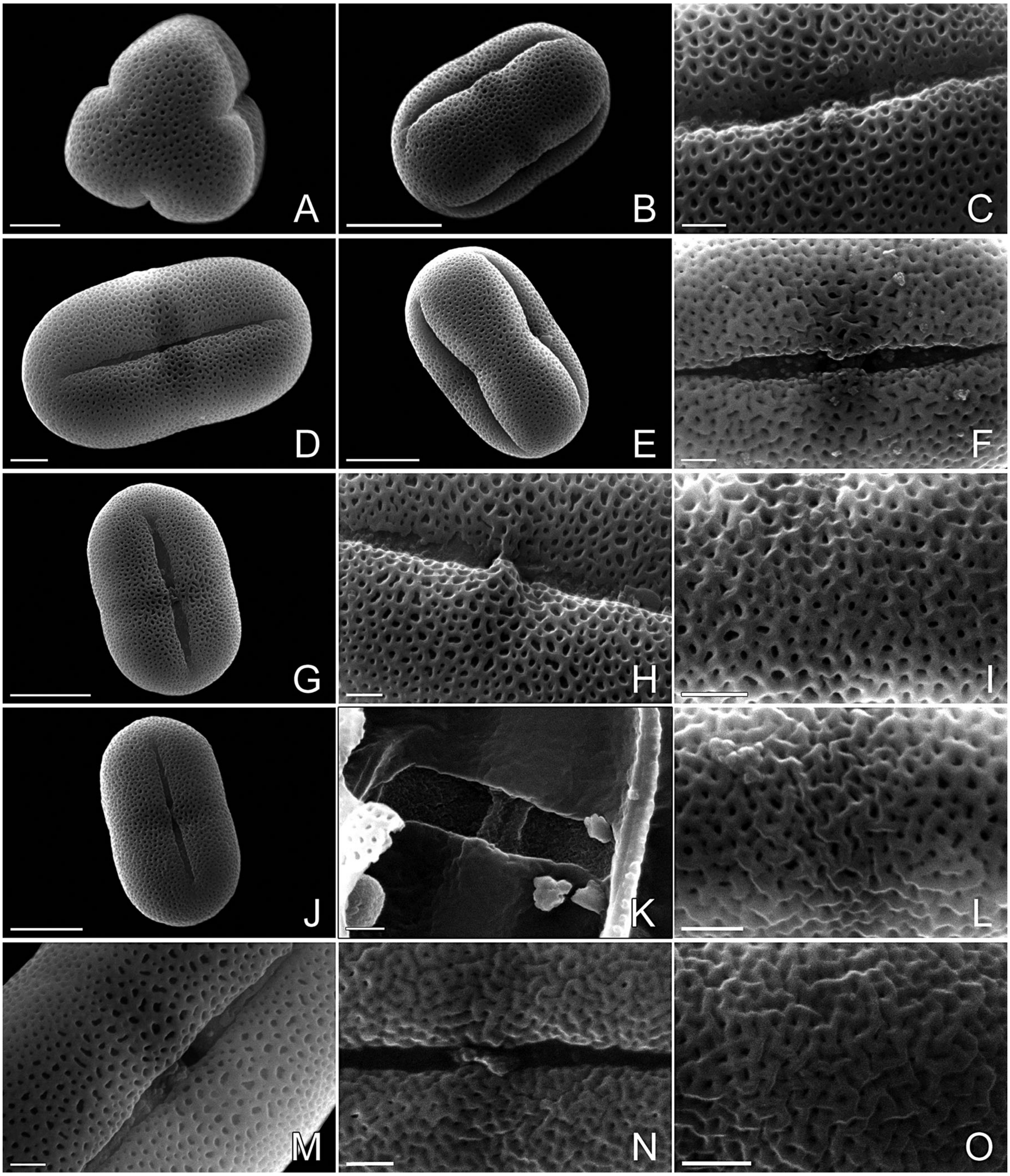

Fig. 5. Scanning electron micrographs of pollen grains from *Androsace* sect. *Chamaejasme*. **A–C,** *Androsace barbulata*. **A,** Polar view. **B,** Equatorial view. **C,** Detail of colpi. **D, E,** *A. bisulca* var. *aurata*. **D,** Equatorial view. **E,** Equatorial view. **F,** *A. villosa*, detail of colpi. **G,** *A. stenophylla*, equatorial view. **H,** *A. sarmentosa*, detail of colpi and bridge. **I,** *A. ovczinnikovii*, detail of exine ornamentation at mesocolpium. **J,** *A. nortonii*, equatorial view. **K,** *A. chamaejasme* subsp. *lehmanniana*, detail of endoaperture. **L,** *A. incana*, detail of exine ornamentation at mesocolpium. **M,** *A. spinulifera*, detail of colpi. **N, O,** *A. mollis*. **N,** Detail of colpi. **O,** Detail of exine ornamentation at mesocolpium. Scale bar = 5 μm (B, E, G, J), 2 μm (A, D), or 1 μm (C, F, H, I, K–O).

et al. (2004); proved to be an independent lineage in the molecular studies of Boucher et al. (2012) and de Vos et al. (2014) (Fig. 9); following the clade designation of Schneeweiss et al. (2004); this may be called /*Orthocaulon*. Here, the combined characteristics of prolate shape, microreticulate exine, and circular polar view support the idea that *A. erecta* is a distinct lineage (Figs. 3I–3L). Some authors also put *A. erecta* into its own section, *Orthocaulon*, on account of its peculiar erect stem (Handel-Mazzetii, 1927; Hu & Yang, 1986; Hu & Kelso, 1996).

Androsace sect. *Aretia*, *A. triflora* Adans. (sect. *Chamaejasme*), *A. chaixii* Gren. & Godr. (sect. *Andraspis*), *Douglasia*,

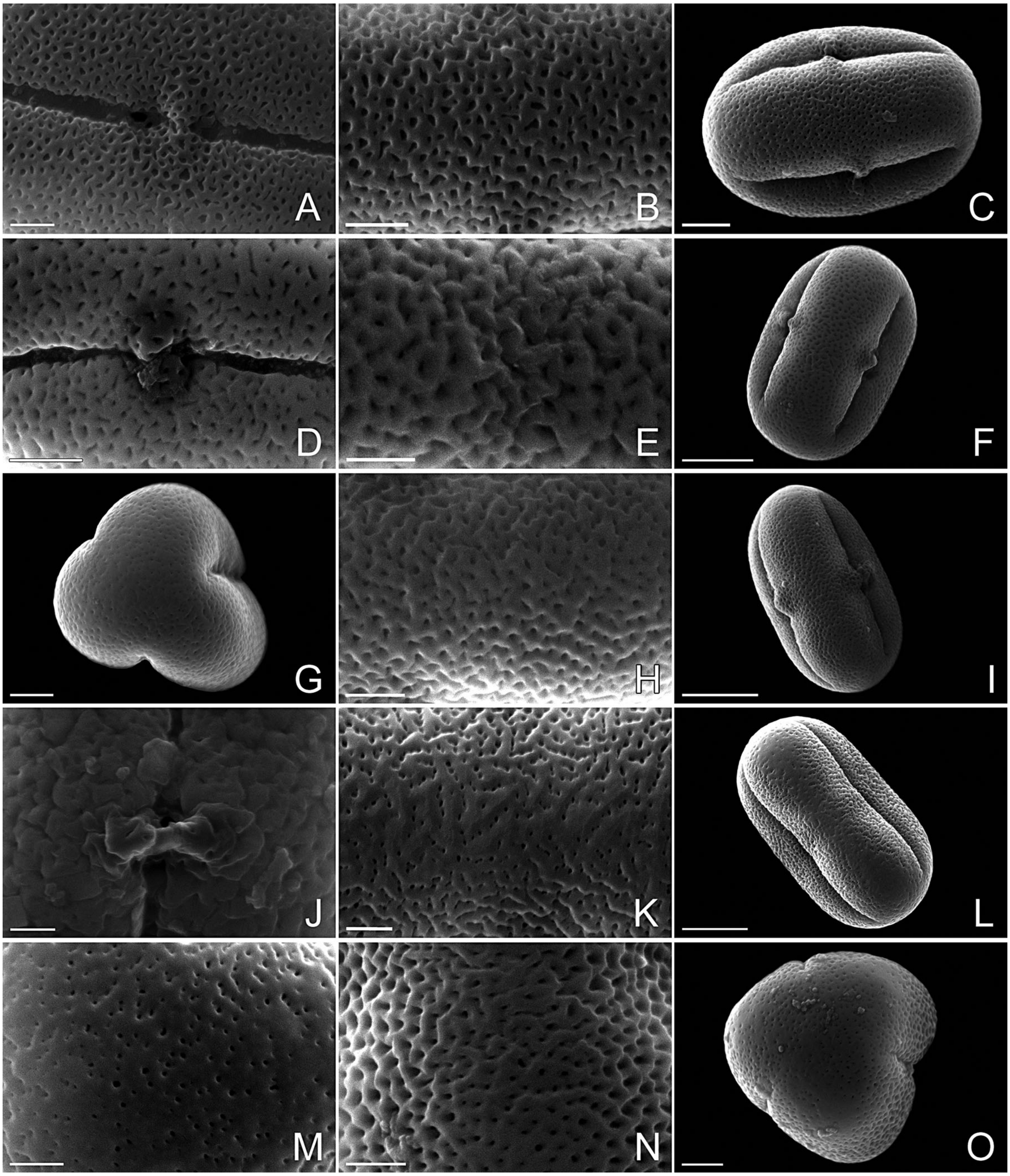

Fig. 6. Scanning electron micrographs of pollen grains from *Androsace* sect. *Aretia* and sect. *Aizoidium*. **A–C,** *Androsace alpina*. **A,** Detail of colpi and bridge. **B,** Detail of exine ornamentation at mesocolpium. **C,** Equatorial view. **D–G,** *A. carnea*. **D,** Detail of colpi. **E,** Detail of exine ornamentation at mesocolpium. **F,** Equatorial view. **G,** Polar view. **H, I,** *A. obtusifolia*. **H,** Detail of exine ornamentation at mesocolpium. **I,** Equatorial view. **J,** *A. wulfeniana*, detail of bridge. **K–M,** *A. bulleyana*. **K,** Detail of exine ornamentation at mesocolpium. **L,** Equatorial view. **M,** Detail of exine ornamentation at apocolpium. **N, O,** *A. integra*. **N,** Detail of exine ornamentation at mesocolpium. **O,** Polar view. Scale bar = 5 μm (F, I, L), 2 μm (C, D, G, O), or 1 μm (A, B, E, H, J, K, M, N).

and *Vitaliana* are all part of /*Aretia* (Schneeweiss et al., 2004; Fig. 9), a species-rich clade that has abundant variation in pollen morphology. There are two basic characteristic patterns (represented by *Douglasia* and *Vitaliana*) within this clade. *Douglasia* species (Figs. 8A–8L) have the largest pollen grains (>20 μm, except *D. montana* A. Gray). *Vitaliana* can be delimited by the prolate shape, perforate exine, and circular polar view (Fig. 9). *Vitaliana* is unique in the group in having heterostyly, and has often been treated as a separate genus (Ferguson, 1972; Smith & Lowe, 1997), although different from

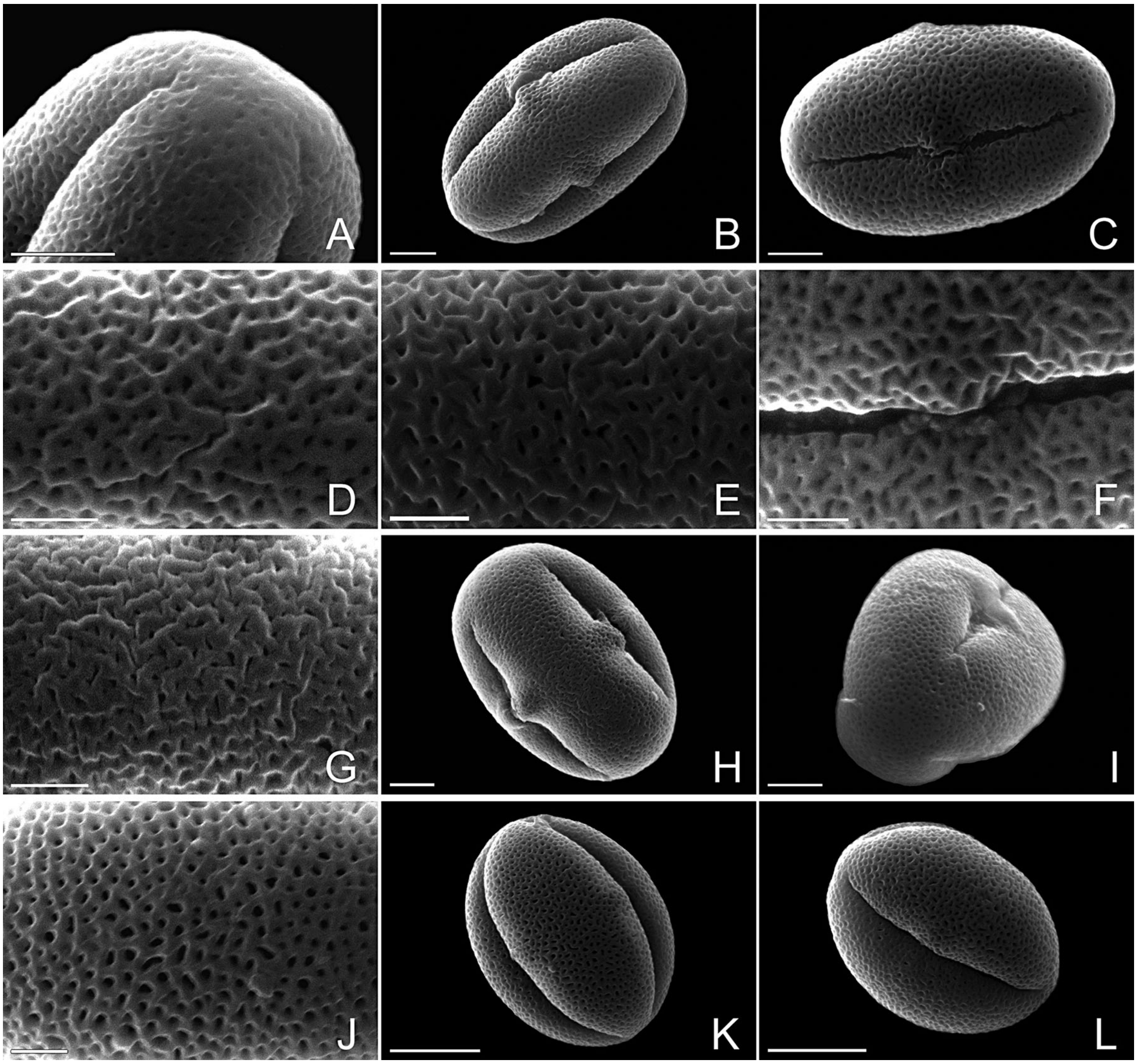

Fig. 7. Scanning electron micrographs of pollen grains from *Androsace* sect. *Androspis*. **A–D,** *Androsace septentrionalis*. **A,** Oblique polar view. **B,** Equatorial view. **C,** Equatorial view. **D,** Detail of exine ornamentation at mesocolpium. **E, F,** *A. septentrionalis* var. *breviscapa*. **E,** Detail of exine ornamentation at mesocolpium. **F,** Detail of colpi. **G–I,** *A. raddeana*. **G,** Detail of exine ornamentation at mesocolpium. **H,** Equatorial view. **I,** Oblique polar view. **J–L,** *A. filiformis*. **J,** Detail of exine ornamentation at mesocolpium. **K,** Equatorial view. **L,** Oblique equatorial view. Scale bar = 5 μm (K, L), 2 μm (A–C, H, I), or 1 μm (D–G, J).

Primula. There is no evidence that heterostyly in *Vitaliana* is associated with dimorphic pollen (Wendelbo, 1961; Halbritter, 2005).

The monophyly of the annual taxa (which belong to *Androsace* sect. *Andraspis*; Fig. 7) is not supported by molecular data, and except for *A. chaixii* (which belongs to /*Aretia*), four annual clades can be distinguished on the basis of pollen characteristics (Schneeweiss et al., 2004; Wang et al., 2004; Boucher et al., 2012; de Vos et al., 2014; Fig. 9; the fifth clade, /*Elongata*, could not be investigated here). Clade /*Megista* (Schneeweiss et al., 2004) includes *A. maxima*, which is distinctive in its pollen morphology (characterized by microechinate exine ornamentation and angulaperturate polar view; Fig. 9). Clade /*Megista* also has been put into its own section *Megista* (Obchinnikov, 1952; Kuvajev & Pirozhkova, 1987), based on its accrescent calyx. However, the pollen morphology of other clades of the short-lived lineages is homogeneous and unique in sect. *Andraspis*, but overlaps with other subgroups. Clade /*Septentrionalis* (Schneeweiss et al., 2004; Figs. 7A–7I, 9) has rugulate and prolate grains, whereas /*Filiformis* (Schneeweiss et al., 2004; Figs. 7J–7L, 9) has microreticulate and spheroidal grains.

Pomatosace is morphologically distinguished from *Androsace*, in having a circumscissile capsule, and a close relationship between these two genera was also indicated by karyological data (Kong & Liu, 1999). Previous palynological studies already showed that *Pomatosace* has the same pollen type as *Androsace* (Wendelbo, 1961; Spanowsky, 1962). Our investigation confirms this view, although it also points out that the pollen morphotype of *Pomatosace* differs from that of other subgroups of *Androsace* in having subprolate shape,

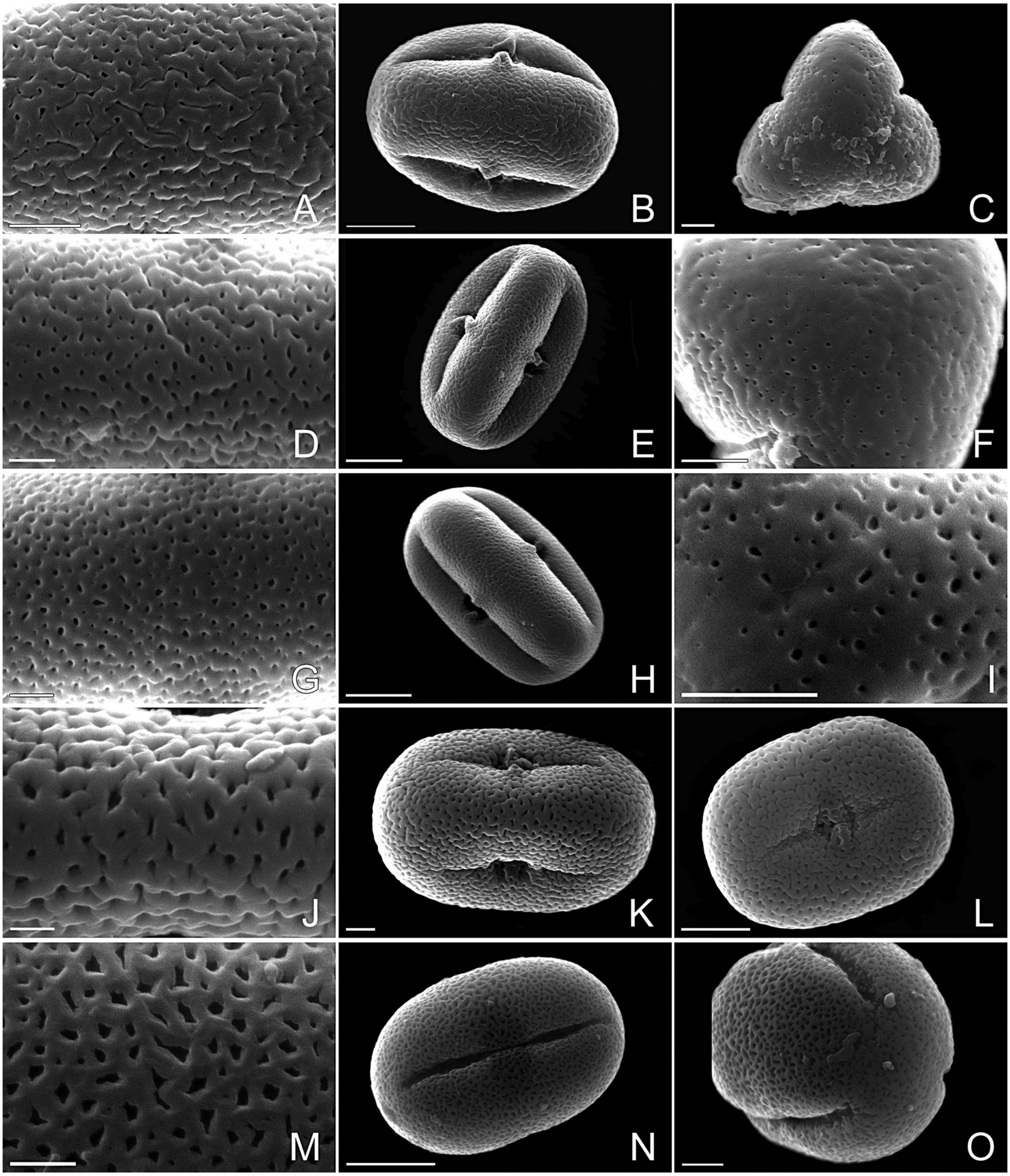

Fig. 8. Scanning electron micrographs of pollen grains from *Douglasia* and *Pomatosace*. **A, B,** *D. arctica*. **A,** Detail of exine ornamentation at mesocolpium. **B,** Equatorial view. **C–F,** *D. laevigata*. **C,** Polar view. **D,** Detail of exine ornamentation at mesocolpium. **E,** Equatorial view. **F,** Detail of exine ornamentation at apocolpium. **G–I,** *D. montana*. **G,** Detail of exine ornamentation at mesocolpium. **H,** Equatorial view. **I,** Detail of exine ornamentation at apocolpium. **J–L,** *D. nivalis*. **J,** Detail of exine ornamentation at mesocolpium. **K,** Equatorial view. **L,** Equatorial view. **M–O,** *Pomatosace filicula*. **M,** Detail of exine ornamentation at mesocolpium. **N,** Equatorial view. **O,** Oblique polar view. Scale bar = 5 μm (B, E, H, L, N), 2 μm (A, C, F, I, K, O), or 1 μm (D, G, J, M).

microreticulate exine with irregular lumina, and circular polar view (Figs. 8M–8O, 9). Recent molecular phylogenetic studies similarly support merging *Pomatosace* into *Androsace*, as a separate lineage, /*Pomatosace* (Schneeweiss et al., 2004; Wang et al., 2004; Boucher et al., 2012; de Vos et al., 2014).

Conclusion

Pollen grains of *Androsace* and related genera are largely homogeneous, and the monophyly of the *Androsace* group is supported by pollen data. Pollen morphology also supports the inclusion of *Douglasia* and *Vitaliana* in this group, but it is

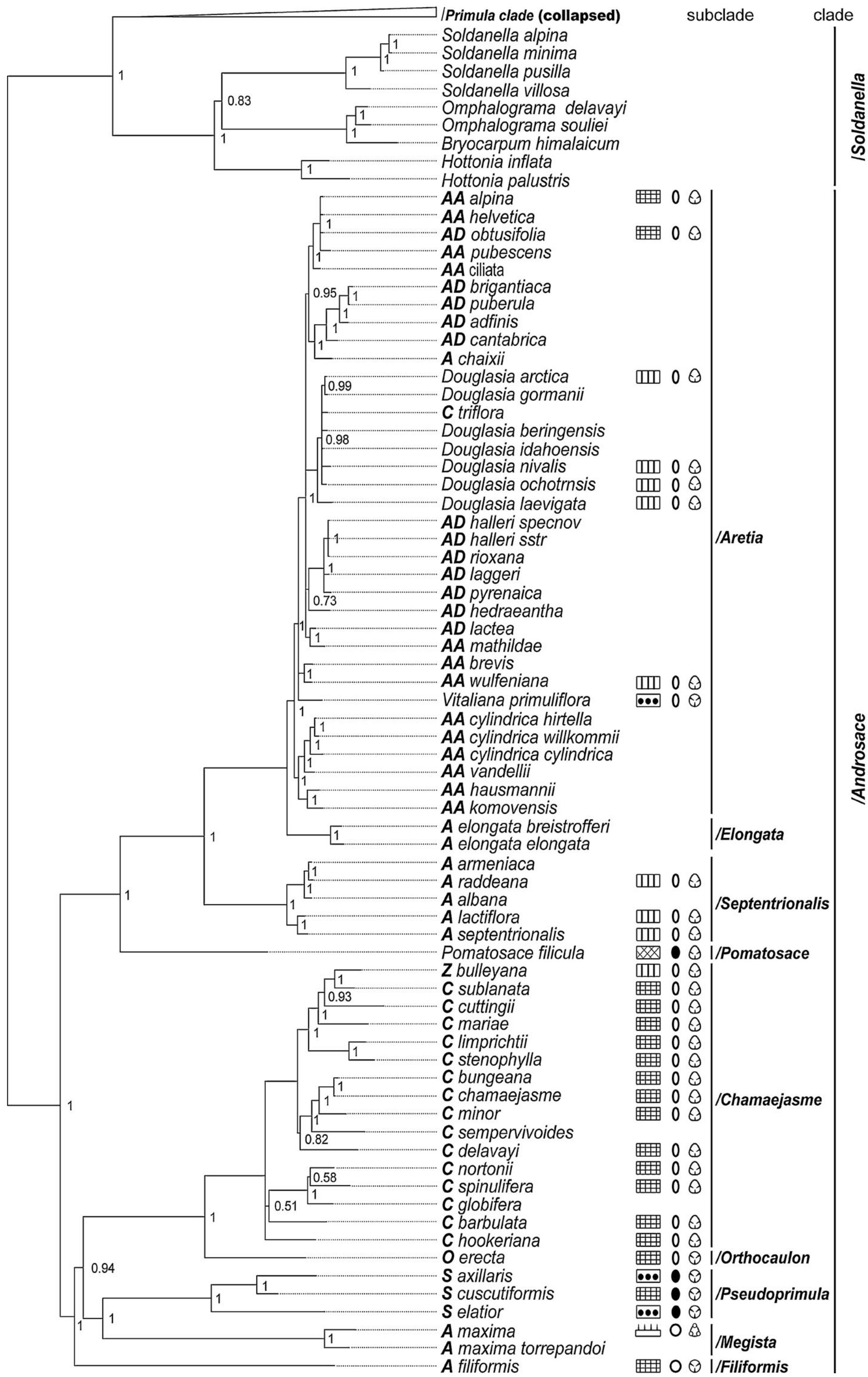

Fig. 9. Pollen characters (exine ornamentation, shape, and polar view) mapped on the Bayesian tree of *Androsace* and related genera inferred using plastid data (*mat*K, *rpl*16, *trn*L, and *trn*L-*trn*F; adapted from de Vos et al., 2014). Abbreviations before names indicate current taxonomic assignment of species to the sections of *Androsace*: *A* = sect. *Andraspis*; *AA* = sect. *Aretia* subsect. *Aretia*; *AD* = sect. *Aretia* subsect. *Dicranothrix*; C = sect. *Chamaejasme*; O = sect. *Orthocaulon*; S = sect. *Samuelia*; Z = sect. *Aizoidium*. The circumscription of clades follows Schneeweiss et al. (2004) with the exception of /*Orthocaulon* introduced here. Symbols after names represent different characters and character-states of pollen grains. The first column represents exine ornamentation: ▦, microreticulate with circular shape of lumina; ⊠, microreticulate with irregular shape of lumina; ▥, rugulate; ●●●, perforate; ⊥⊥⊥, microechinate. The second column represents the shape of the pollen grain: O, spheroidal (P/E = 0.88–1.14); ●, subprolate (P/E = 1.14–1.33); 0, prolate (P/E = 1.33–2.00). The third column represents polar view type: ○, circular and peritreme; △, triangular and planaperturate; △, triangular and angulaperturate.

unable to resolve currently recognized taxonomic groups within the *Androsace* group because of some overlapping characters among these taxa. However, combined pollen morphological characters based on shape, exine ornamentation, and polar view support the demarcation of several clades recognized by molecular phylogenetic analyses, including /*Septentrionalis*, /*Pomatosace*, /*Orthocaulon*, and /*Megista*. More in-depth analyses of morphology and expanded molecular phylogenetic studies with more extensive sampling of taxa and molecular markers will be needed to obtain a more robust and better resolved phylogeny of the *Androsace* group.

Acknowledgements

We thank IBSC, PE, and US for herbarium materials, Wei-Tao Jin and Nan-Cai Pei for collecting pollen samples, and Xiao-Ying Hu for technical assistance. We also thank James Doyle and Editor Sean Graham for their valuable comments. This study was financially supported by the National Natural Science Foundation of China (Grant No. 31270260).

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