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Pollen morphology of tribes Alsineae and Sperguleae (Caryophyllaceae) and its systematic significance

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ABSTRACT

Pollen morphology of 79 species, one subspecies and eight varieties representing nine genera of tribe Alsineae and two of tribe Sperguleae was studied using light microscopy (LM) and scanning electron microscopy (SEM). Among them, 53 species and eight varieties (22 species of which are endemic to China) were reported for the first time. The results demonstrate that the number of pores, the pollen surface ornamentation and the density of microechini have important systematic significance. The surface ornamentation can be divided into three types, namely microechinate-perforate, microechinate-punctate, and microechinate-punctate-perforate. Pollen characteristics support: (i) isolation of *Arenaria* subgenus *Odontostemma* from *Arenaria* and reclassification of *Arenaria* subgenus *Odontostemma* to *Odontostemma*; (ii) isolation of *Cerastium* subgenus *Dichodon* from *Cerastium* and reclassification of *Cerastium* subgenus *Dichodon* to *Dichodon*; (iii) isolation of *Minuartia* subgenus *Rhodalsine* from *Minuartia* and reclassification of *Minuartia* subgenus *Rhodalsine* to *Rhodalsine*; (iv) isolation of *Rhodalsine* from tribe Alsineae and reclassification of *Rhodalsine* into tribe Sperguleae; and (v) isolation of *Sagina* from tribe Alsineae. In addition, the relationships of *Arenaria* subgenus *Eremogone* and *Arenaria* subgenus *Eremogoneastrum* to tribe Alsineae need to be further studied. Furthermore, pollen characteristics indicate that tribe Alsineae is more evolved than tribe Sperguleae.

KEYWORDS
Alsineae; pollen morphology; Sperguleae; systematics

1. Introduction

The family Caryophyllaceae belongs to the order Caryophyllales (APG IV 2016). It contains about 100 genera and 3000 species, which are mainly distributed in the northern temperate zone with diversified centres located in the Mediterranean and Irano–Turanean region (Hernández-Ledesma et al. 2015). In traditional taxonomy, according to the types of its stipules, petals, fruits, and embryonic development, Caryophyllaceae is classified into subfamilies Alsinoideae, Caryophylloideae and Paronychioideae. Tribe Alsineae belongs to subfamily Alsinoideae, and tribe Sperguleae belongs to subfamily Paronychioideae (Bittrich 1993; Tang et al. 1996).

Different scholars have classified tribe Alsineae differently. Pax and Hoffmann (1934) classified tribe Alsineae into subtribe Sabulininae and subtribe Stellariinae according to the way their capsules crack.

McNeill (1962) divided the tribe Alsineae into three groups: the *Arenaria* group containing *Arenaria* L., *Brachystemma* D.Don, *Bufonia* L., *Gooringia* Williams, *Honckenya* Ehrh., *Lepyrodiclis* Fenzl, *Minuartia* L., *Moehringia* L., *Reicheella* Pax, *Thylacospermum* Fenzl, *Thurya* Boiss. & Balansa and *Wilhelmisa* Rchb.; the *Stellaria-Cerastium* group comprising *Cerastium* L., *Myosoton* Moench, *Holosteum* L., *Moenchia* Ehrh., *Pseudostellaria* Pax, and *Stellaria* L.; and the *Sagina* group containing *Sagina* L. and *Colobanthus* Bartl.

Bittrich (1993) believed that according to the presence of nectaries in the stamen and the type of fruit, tribe Alsineae should contain 23 genera, namely *Alsinidendron* H. Mann, *Arenaria*, *Thylacospermum*, *Moehringia*, *Brachystemma*, *Bufonia*, *Cerastium*, *Colobanthus*, *Holosteum*, *Honckenya*, *Lepyrodiclis*, *Minuartia*, *Moenchia*, *Myosoton*, *Plettkea* Mattf., *Pycnophyllopsis* Skottsb., *Pseudostellaria*, *Reicheella*, *Sagina*, *Schiedea* Cham. & Schlchtd., *Stellaria*, *Thurya* and *Wilhelmisa*.

Tribe Sperguleae includes four genera, namely *Rhodalsine* J.Gay, *Sanctambrosia* Skottsb., *Spergula* L. and *Spergularia* (Pers.) J.Presl & C.Presl, and about 51 species (Kool 2012; Kool and Thulin 2017a). Among them, *Rhodalsine* has been placed in the tribe Alsineae as *Minuartia* subgenus *Rhodalsine* (McNeill 1962; Bittrich 1993).

Recent phylogenetic studies have proposed to abolish the view that Caryophyllaceae is divided into three subfamilies (Harbaugh et al. 2010; Greenberg and Donoghue 2011) and proven that tribe Alsineae is not a monophyletic group and should be re-classified (Smissen et al. 2002; Fior et al. 2006; Fior and Karis 2007). In addition, the infrageneric classification of tribe Alsineae is controversial. For example, the definition of *Arenaria* has been discrepant (Fenzl 1840, 1842; Bentham and Hooker 1862; Williams 1895; McNeill 1962; Rabeler and Hartman 2005; Sadeghian et al. 2014, 2015). The intrageneric classification of *Cerastium* has also drawn significant attention (Schischkin 1970; Czerepanov 1995; Scheen et al. 2004; Arabi

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et al. 2017, 2018). *Minuartia* has been proven to not be a monophyletic group, and its infrageneric classification is inconclusive (Löve and Löve 1975; Dillenberger and Kadereit 2014).

The species origin, taxonomy and phylogenetic relationships of tribes Alsineae and Sperguleae have been studied from the aspects of seed morphology (Echlin 1968; Wofford 1981; Wyatt 1984; Minuto et al. 2006; Casazza and Minuto 2008; Memon et al. 2010; Minuto et al. 2011; Mostafavi et al. 2011, 2013; Kanwal et al. 2012; Koç et al. 2012; Sadeghian et al. 2014; Lorite et al. 2018; Ullah, Zafar, Amhad, Sultana et al. 2018), plant external morphology (Brysting and Elven 2000; Conti 2003; Koç et al. 2019), anatomy (Schweingruber 2007; Mousavi et al. 2019), cell biology (Boşcaiu et al. 1999), biogeography (Moore et al. 2013; Iamonico 2015) and molecular biology (Brysting et al. 2011; Moore and Kadereit 2013; Dillenberger and Kadereit 2017).

Pollen is the male gametophyte of plants. Compared with other morphological characteristics such as roots, stems, leaves, etc., it has high genetic stability. The component sporopollenin of its exine is resistant to acid and alkali treatments (Erdtman 1969; Wang et al. 1997). The morphological characteristics of pollen grains are not only widely used in the fields of palaeobotany, palaeoecology and atmospheric palynology, but also play important roles in revealing species origin, phylogenetic relationships and plant taxonomy (Walker and Doyle 1975; Sahreen et al. 2008; Tuler et al. 2017; Song et al. 2018; Gul et al. 2019; Ullah, Zaman et al. 2019).

Pollen morphology (such as number of pores, density of microechini, surface ornamentation and exine thickness) of tribes Alsineae and Sperguleae has been widely used in taxonomy at various levels, including intergeneric and intrageneric, interspecies and intraspecies classifications (Chanda 1962; McNeill and Basset 1974; Taia 1994; Yıldız 2001; Perveen and Qaiser 2006).

Although many researchers have studied the pollen grains of tribes Alsineae and Sperguleae, they only focused on description of pollen morphological features of a few species and genera, and some studies only described some of the pollen characteristics (Erdtman et al. 1961; Erdtman 1968; Nowicke 1975; Skvarla and Nowicke 1976; Iwarsson 1977; Al-Eisawi 1989; Diaz de la Guardia et al. 1991; Parent and Richard 1993; Punt and Hoen 1995; Dang et al. 1998; Chen et al. 2009; Bozchaloyi and Keshavarz 2014).

Candau (1978) studied the diameter, shape and surface ornamentation of pollen grains, as well as the aperture diameter of 37 species of nine genera of tribe Alsineae in subfamily Alsinoideae and classified the pollen surface ornamentation of tribe Alsineae as microechinate and microperforate. But he did not describe the aperture spacing, the number of granules on the operculum, or the density of microechini.

Dang and Jiang (1995) studied the pollen morphology of 12 species of three genera of the tribe Alsineae in China and found that their pollen grains are spheroidal or nearly spherical and have pantoporate apertures with warty granules, a protruding or depressed membrane, and granulate surface ornamentation with small grooves.

Dang and Jiang (1996) studied the pollen morphology of two species of *Spergularia* in China, and believed that their pollen grains are oblong in the polar view and spherical in the equatorial view, with a tricolpate aperture and granulate surface ornamentation.

Perveen and Qaiser (2006) studied the pollen morphology of 74 species of 23 genera of Caryophyllaceae in Turkey, including 19 species of six genera in tribe Alsineae, and five species of two genera in tribe Sperguleae. They described the pollen size and shape, and divided the pollen surface ornamentation into spinulose-punctate and scabrate-punctuate types. However, they did not describe the spacing and number of apertures or the number of granules on the operculum, nor did they discuss the relevant taxonomic issues.

Mostafavi and Mehregan (2014) studied the pollen of 20 species of *Minuartia* in Iran and found that their pollen grains are nearly spherical or polyhedral, and have microechinate and microechinate-punctate surface ornamentations.

Wan and Liu (2017) studied the pollen of 64 species and five varieties of *Stellaria* in China and found that their pollen grains are spherical or spherical-polyhedral, with pantoporate apertures and microechinate-perforate, microechinate-punctate and microechinate-punctate-perforate surface ornamentations.

Doğan and Ömer Erdem (2018) studied the pollen of 11 taxa belonging to *Arenaria* and found that their pollen grains are oblong with pantoporate apertures and microechinate-perforate surface ornamentation.

Ullah, Shah et al. (2019) described the pollen of 18 species of seven genera of subfamily Alsinoideae from Pakistan and found that their pollen grains with echini on the surface are subspheroidal or prolate and have microechinate-punctate or microechinate-perforate surface ornamentation.

In this study, the pollen morphology of nine genera including 77 species, one subspecies and eight varieties of the tribe Alsineae and two genera including two species of the tribe Sperguleae are observed and studied in detail using light microscopy (LM) and scanning electron microscopy (SEM). Among them, 45 species, one subspecies and five varieties are found in China and 22 species are endemic to China. In addition, the pollen morphology of 53 species and eight varieties is reported for the first time. This study aims to provide palynological information for the taxonomic and phylogenetic study of tribes Alsineae and Sperguleae.

2. Materials and methods

2.1. Experimental materials

Pollen grains of the studied species were collected from the herbarium of the South China Botanical Garden, Chinese Academy of Sciences (IBSC) and the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). The voucher information for all specimens is given in Table S1 (in the supplementary materials). In addition, the species were confirmed on the basis of characters mentioned in the original descriptions of Flora of China and The Plant List. Their names are accepted in Tropicos and the International Plant Names Index (IPNI). The geographical distribution of the samples is marked on the map presented in Figure 1.

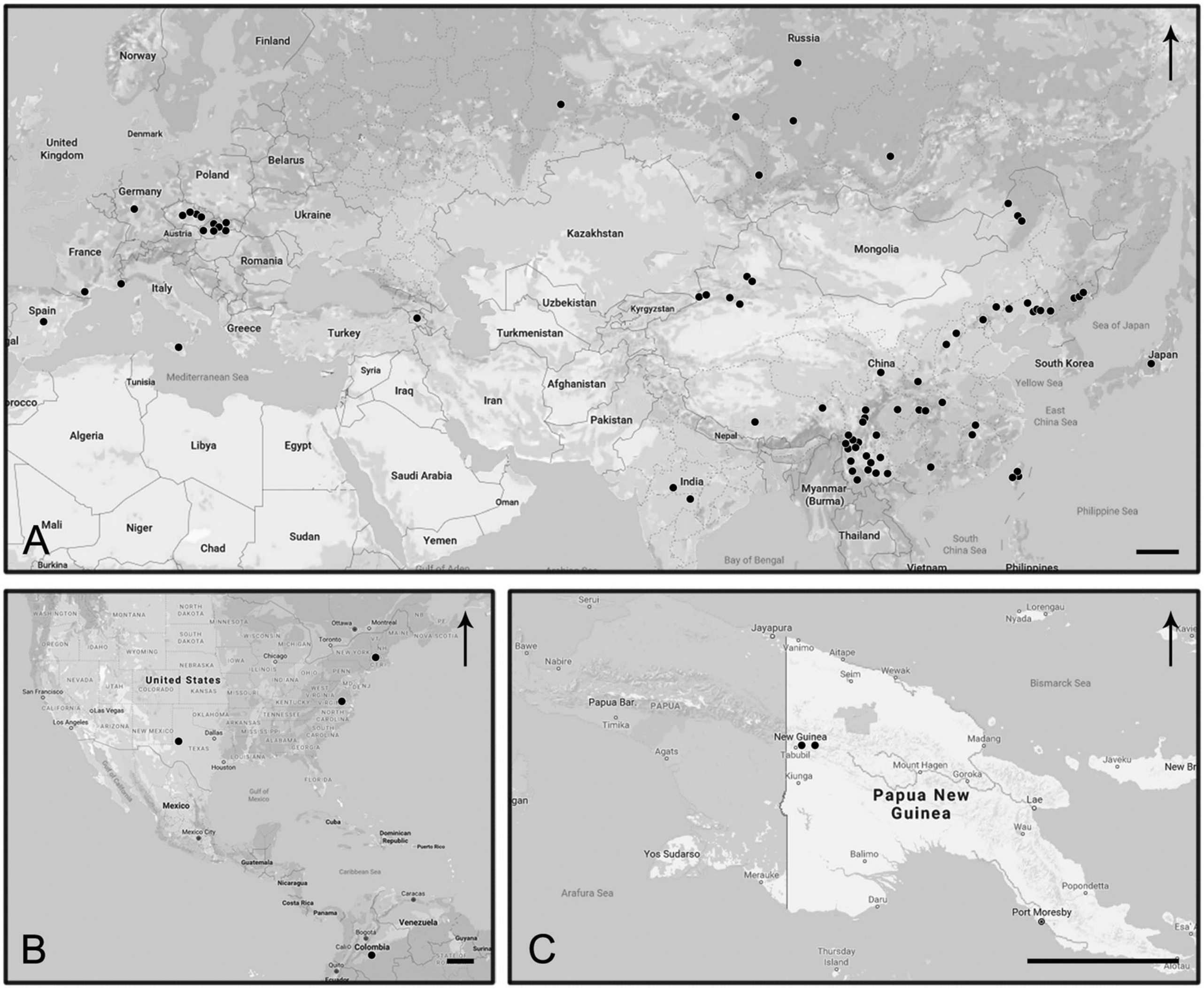

Figure 1. Occurrence map of the analysed species of tribes Alsineae and Sperguleae, showing the distribution of sample locations in (A) Asia and Europe, (B) America and (C) New Guinea. Map data © 2019 Google Maps. Scale bar: 400 km.

2.2. Methods

The naturally dried pollen grains were prepared for LM by acetolysis (Erdtman 1969), then mounted in glycerin jelly. All measurements were taken within 7 days of preparation (Melhem and Matos 1972; Salgado-Labouriau 1973). Images were captured using a Leica DM6000 upright fluorescence microscope.

For the SEM study, pollen grains were not subjected to acetolysis, to preserve the exine and intine (Harley and Ferguson 1990; Halbritter and Hesse 2004). The non-acetolysed pollen grains were scattered evenly on aluminium stubs covered with double-sided transparent adhesive tape. All samples were coated with gold-palladium, examined under a Hitachi S-4800 scanning electron microscope at 10 kV and photographed.

Twenty-five pollen grains of each species were randomly selected, and their diameter, the diameter of the pores or the colpus length and width, the distance between two pores, the exine thickness and the density of microechini (the number of microechini in a 5 μm × 5 μm area of pollen grain surface) were measured. Statistical analyses were conducted to obtain arithmetic means, standard deviations and 95% confidence interval (CI 95%). To compare the values of the pollen grain diameter, we used the graphs of SPSS version 22.0 (IBM SPSS Statistics), which represent the mean and the confidence interval values.

Palynological terminology is used from previous works (Erdtman 1969; Wang et al. 1997; Punt et al. 2007; Hesse et al. 2009).

3. Results

The detailed pollen characteristics of all examined samples are listed in Tables S2–S4 (supplementary materials) and Plates 1–17. The pollen characteristics of each genus are described as follows.

3.1. *Arenaria*

Arenaria contains about 300 species worldwide. Among them, 102 species are found in China, 79 of which are endemic to China (Wu et al. 2001). In this study, 30 species and one variety are investigated, of which 26 species and one variety are reported for the first time.

Arenaria has spherical or spheroidal-polyhedral pollen grains with a diameter of 20.76–40.10 μm, and has 10–18 round pores uniformly distributed on the surface. The diameter of the pores

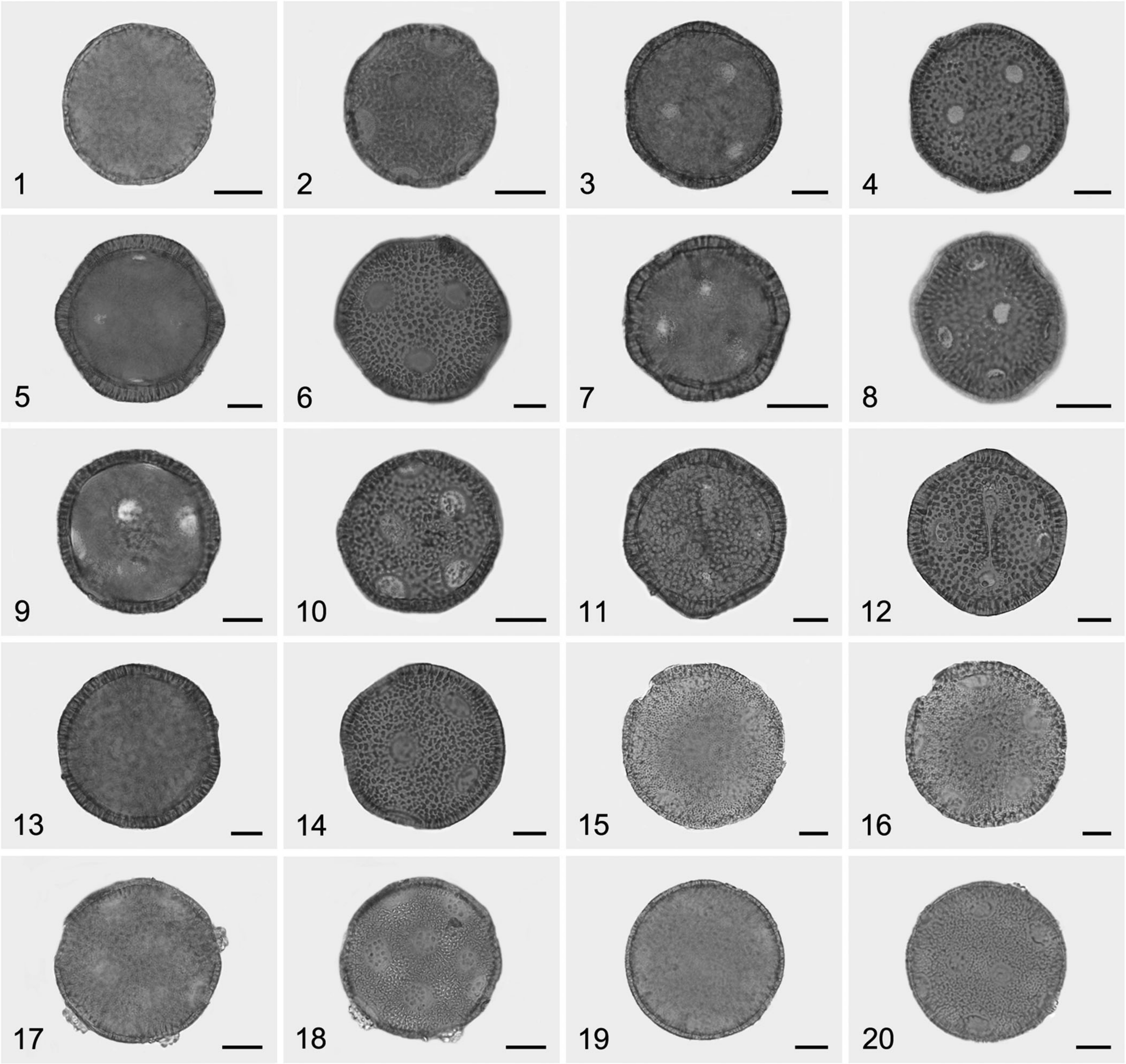

Plate 1. Light microscopy (LM) micrographs of tribe Alsineae pollen grains. 1–2. *Arenaria arundana*; 3–4. *A. atuntziensis*; 5–6. *A. barbata*; 7–8. *A. brevipetala*; 9–10. *A. ciliata*; 11–12. *A. debilis*; 13–14. *A. delavayi*; 15–16. *A. dimorphitricha*; 17–18. *A. erinacea*; 19–20. *A. euodonta*. Scale bar: 10 µm.

is 2.89–5.90 µm and the distance between two pores is 4.56–9.41 µm. There are 5–17 granules on the operculum. Exine thickness is 1.15–3.85 µm. The number of microechini per sample area of 5 µm × 5 µm ranges from 6 to 15. The pollen grains have microechinate-perforate (Plate 10, figures 15–16) microechinate-punctate (Plate 9, figures 17–18) and microechinate-punctate-perforate ornamentation (Plate 10, figures 5–6).

3.2. *Bufonia*

Bufonia includes 20 species worldwide (Bittrich 1993). In this research, we studied the species *Bufonia parviflora* Grisb., and its pollen morphology is reported for the first time.

The pollen grains of *Bufonia* are spheroidal-polyhedral with a diameter of 15.65–18.61 µm, and have 10–12 round pores uniformly distributed on the surface. The diameter of the pores is 2.18–3.57 µm and the distance between two pores is 3.17–5.75 µm. There are 5–10 granules on the operculum. Exine thickness is 1.21–2.41 µm. The number of microechini in a sample area of 5 µm × 5 µm ranges from 11 to 16. The pollen grains have microechinate-punctate ornamentation (Plate 12, figures 3–4).

3.3. *Cerastium*

Cerastium comprises approximately 100 species worldwide. Among them, 29 species are found in China, nine of which are endemic to China (Lu et al. 2001). In this study, 26 species, one subspecies and five varieties are investigated, of which 17 species and five varieties are reported for the first time.

The pollen grains of *Cerastium* are spherical or spheroidal-polyhedral with a diameter of 20.67–35.81 µm, and have

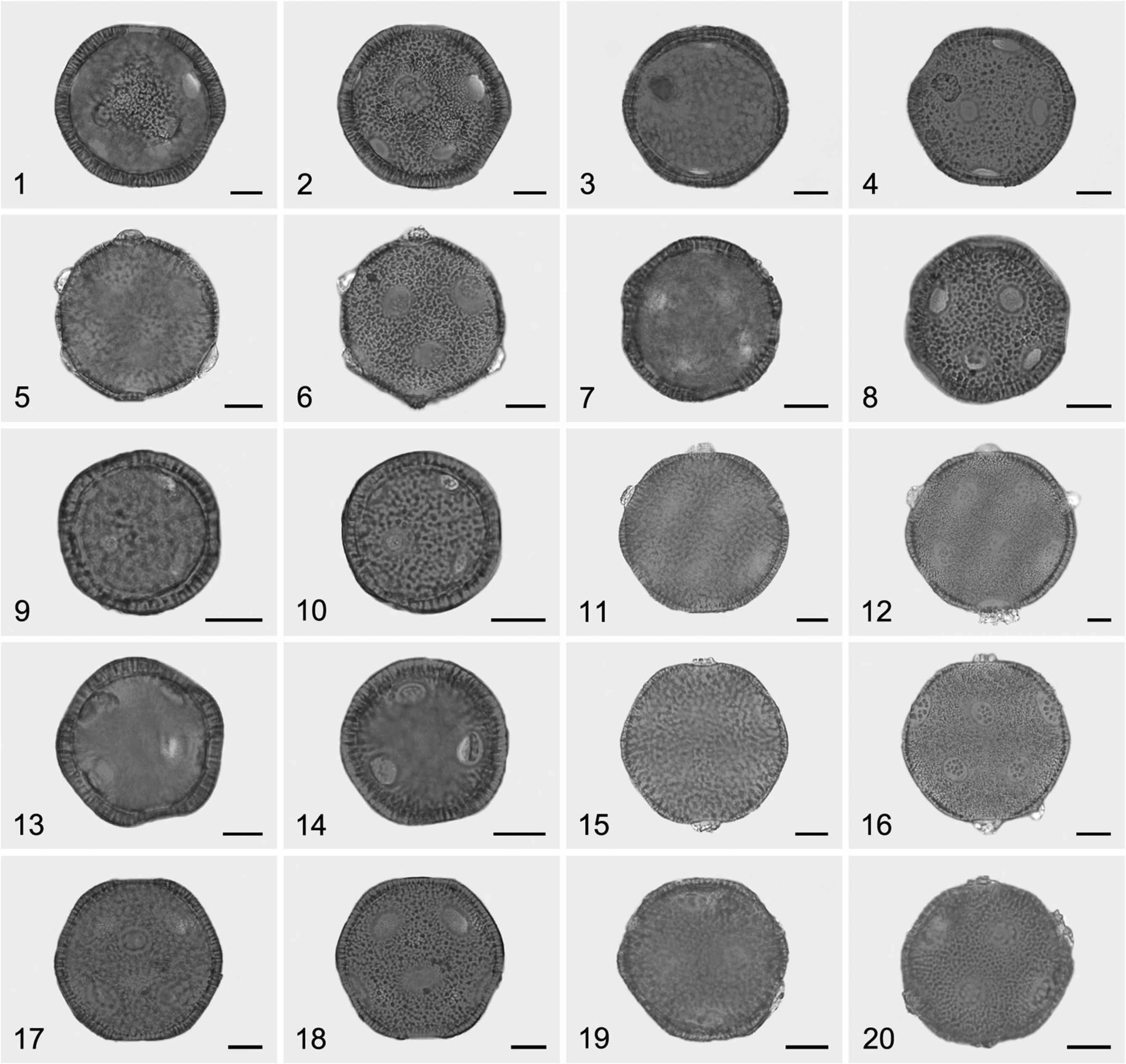

Plate 2. Light microscopy (LM) micrographs of tribe Alsineae pollen grains. 1–2. *Arenaria fimbriata*; 3–4. *A. foliosa*; 5–6. *A. grandiflora*; 7–8. *A. ionandra*; 9–10. *A. juncea* var. *glabra*; 11–12. *A. leucasteria*; 13–14. *A. longifolia*; 15–16. *A. longipetiolata*; 17–18. *A. longistyla*; 19–20. *A. napuligera*. Scale bar: 10 μm.

10–20 round pores uniformly distributed on the surface. The diameter of the pores is 2.36–5.73 μm and the distance between two pores is 4.54–9.50 μm. There are 6–13 granules on the operculum. The exine thickness is 1.12–3.05 μm. The number of microechini per sample area of 5 μm × 5 μm ranges from 7 to 16. The pollen grains have microechinate-perforate (Plate 14, figures 1–2), microechinate-punctate (Plate 13, figures 13–14) and microechinate-punctate-perforate ornamentation (Plate 14, figures 15–16).

3.4. *Holosteum*

Holosteum has about four species worldwide, but only one species in China, namely *Holosteum umbellatum* L. (Lu et al. 2001).

The pollen grains of *Holosteum* are spheroidal-polyhedral with a diameter of 24.55–31.29 μm, and have 16–20 round pores uniformly distributed on the surface. The diameter of the pores is 1.98–4.36 μm, and the distance between two pores is 3.56–7.33 μm. There are 8–17 granules on the operculum. The exine thickness is 1.38–2.59 μm. The number of microechini per sample area of 5 μm × 5 μm ranges from 13 to 18. The pollen grains have microechinate-punctate ornamentation (Plate 15, figures 9–10).

3.5. *Lepyrodiclis*

Lepyrodiclis has about three species worldwide, of which two species are found in China. Here we describe one species,

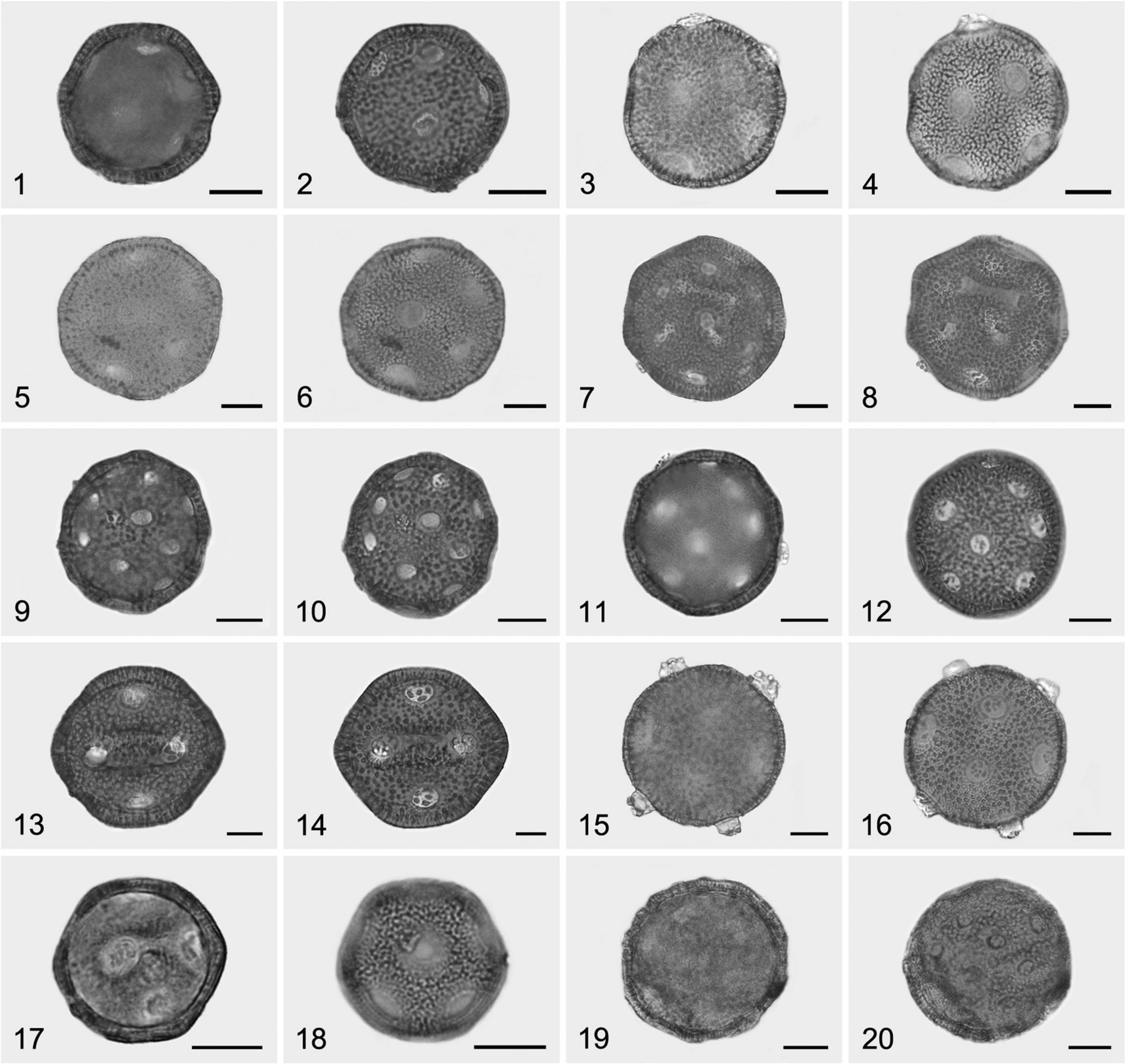

Plate 3. Light microscopy (LM) micrographs of tribe Alsineae pollen grains. 1–2. *Arenaria orbiculata*; 3–4. *A. oreophila*; 5–6. *A. pseudostellaria*; 7–8. *A. quadridentata*; 9–10. *A. serpyllifolia*; 11–12. *A. stenophylla*; 13–14. *A. xerophila*; 15–16. *A. yunnanensis*; 17–18. *Bufonia parviflora*; 19–20. *Cerastium alpinum*. Scale bar: 10 μm.

namely *Lepyrodiclis holosteoides* (C. A. Mey.) Fisch. et Mey. (Lu et al. 2001).

The pollen grains of *Lepyrodiclis* are spheroidal with a diameter of 25.15–30.89 μm, and have 28–32 round pores uniformly distributed on the surface. The diameter of the pores is 1.58–2.77 μm and the distance between two pores is 2.97–5.54 μm. There are 3–8 granules on the operculum. The exine thickness is 1.21–2.59 μm. The number of microechini per sample area of 5 μm × 5 μm ranges from 16 to 22. The pollen grains have microechinate-punctate-perforate ornamentation (Plate 15, figures 11–12).

3.6. *Minuartia*

Minuartia contains about 120 species worldwide. Among them, nine species are found in China (Lu et al. 2001). In this study, 12 species and two varieties are investigated, of which six species and two varieties are reported for the first time.

The pollen grains of *Minuartia* (excluding subgenus *Rhodalsine*) are spheroidal with a diameter of 19.56–29.90 μm, and have 12–20 round pores uniformly distributed on the surface. The diameter of the pores is 1.94–4.35 μm, and the distance between two pores is 4.01–8.14 μm. There are 6–12 granules on the operculum. The exine thickness is 1.07–3.94 μm. The number of microechini per sample area of 5 μm × 5 μm ranges from 12 to 20. The pollen grains have microechinate-perforate (Plate 16, figures 17–18), microechinate-punctate (Plate 16, figures 5–6) and microechinate-punctate-perforate ornamentation (Plate 15, figures 15–16).

The pollen morphology of subgenus *Rhodalsine* is special. Pollen grains of the subgenus *Rhodalsine* are small in size.

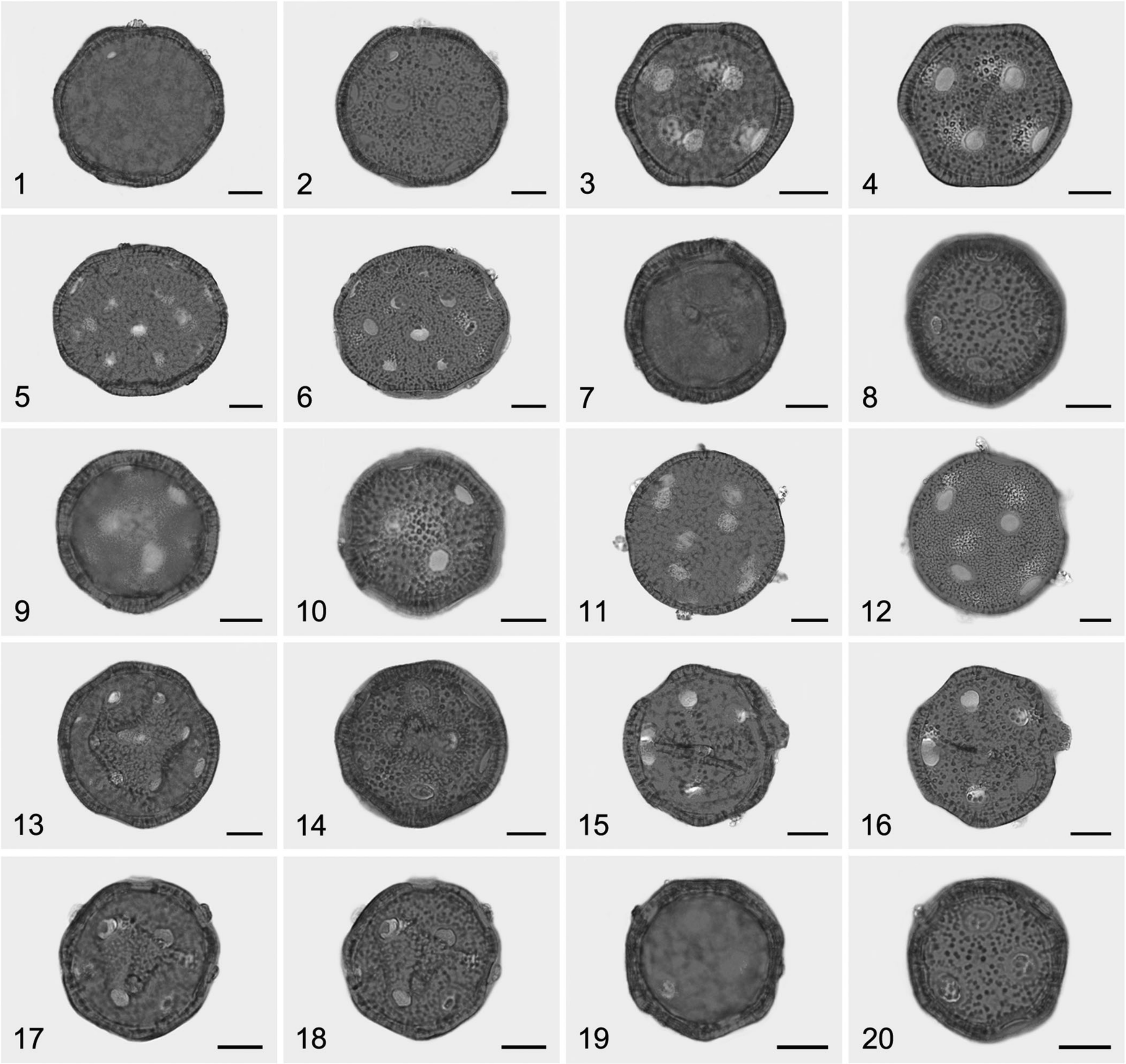

Plate 4. Light microscopy (LM) micrographs of tribe Alsineae pollen grains. 1–2. *Cerastium alsinifolium*; 3–4. *C. anomalum*; 5–6. *C. arvense*; 7–8. *C. baischanense*; 9–10. *C. cerastoides*; 11–12. *C. dahuricum*; 13–14. *C. fischerianum*; 15–16. *C. fontanum*; 17–18. *C. fontanum* subsp. *vulgare*; 19–20. *C. glomeratum*. Scale bar: 10 μm.

The average length of the polar axis (P) is 13.19 (11.96–15.14) μm and the average length of the (E) equatorial axis is 10.72 (7.84–12.85) μm. The P/E ratio is 1.25, the grains being subprolate in shape. They are subcircular in equatorial view and semi-triangular in polar view, and tricolpate. The surface of the colpus membrane is granulate. The exine thickness is 2.58 (1.72–3.53) μm. The number of microechini per sample area of 5 μm × 5 μm ranges from 75 to 125. The pollen grains have microechinate-punctate ornamentation (Plate 15, figures 19–20; Plate 16, figures 1–2).

3.7. *Moehringia*

Moehringia has about 25 species worldwide, of which three species are found in China (Lu et al. 2001). Here we describe one species, namely *Moehringia trinervia* (L.) Clairv.

The pollen grains of *Moehringia* are spheroidal with a diameter of 20.96–25.61 μm, and have 10–14 round pores uniformly distributed on the surface. The diameter of the pores is 2.62–3.69 μm and the distance between two pores is 4.82–7.18 μm. There are 5–10 granules on the operculum. The number of microechini per sample area of 5 μm × 5 μm ranges from 18 to 21. The pollen grains have microechinate-punctate-perforate ornamentation (Plate 17, figures 3–4).

3.8. *Sagina*

Sagina contains about 30 species worldwide. Among them, four species are found in China (Lu et al. 2001). In this study,

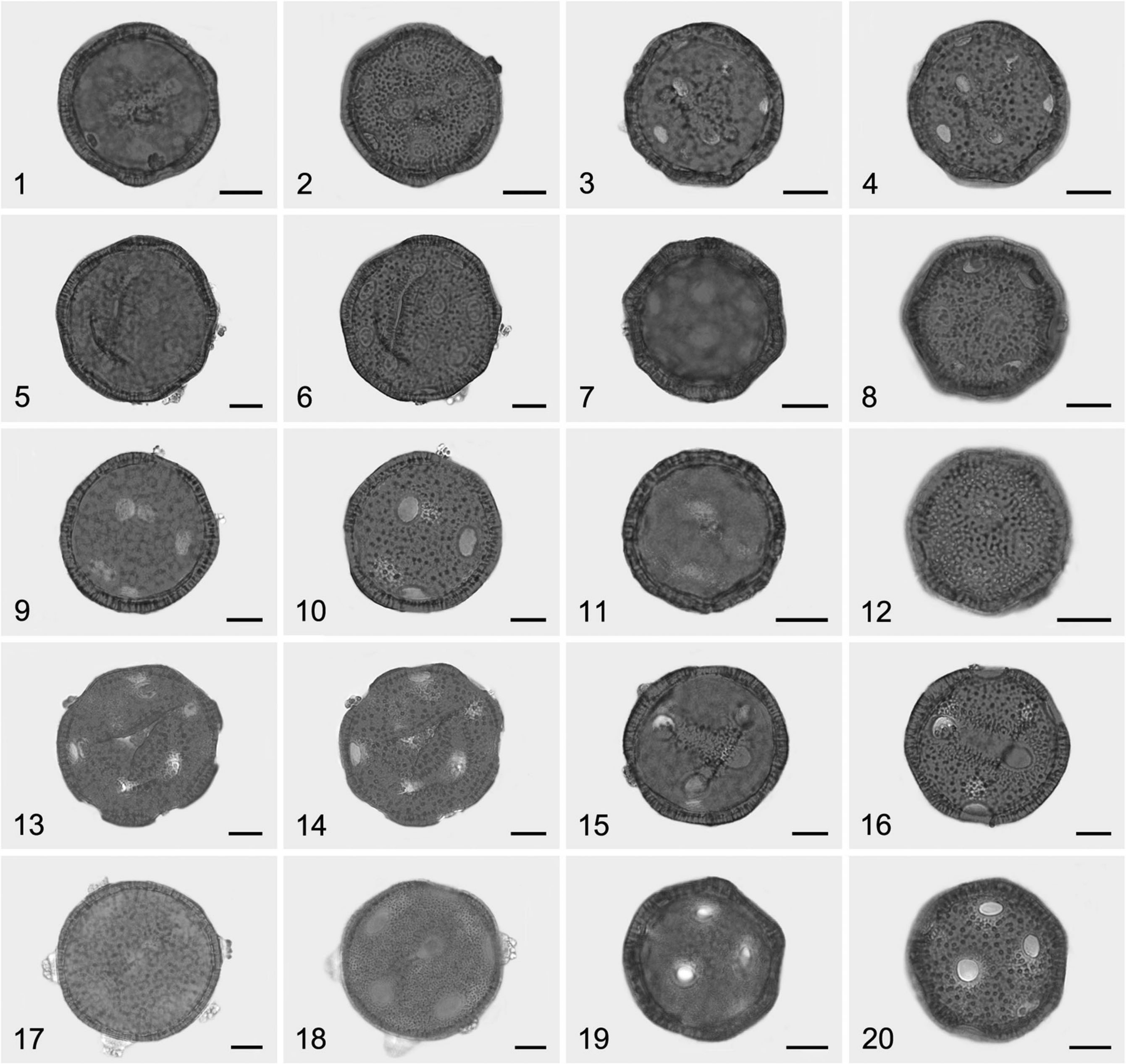

Plate 5. Light microscopy (LM) micrographs of tribe Alsineae pollen grains. 1–2. *Cerastium glutinosum*; 3–4. *C. holosteoides* var. *angustifolium*; 5–6. *C. limprichtii*; 7–8. *C. lithospermifolium*; 9–10. *C. maximum*; 11–12. *C. nutans*; 13–14. *C. oblongifolium*; 15–16. *C. pauciflorum* var. *oxalidiflorum*; 17–18. *C. polymorphum*; 19–20. *C. ponticum*. Scale bar: 10 μm.

four species are investigated, of which two species are reported for the first time.

The pollen grains of *Sagina* are spheroidal or spheroidal-polyhedral with a diameter of 22.80–24.73 μm, and have 24–36 round pores uniformly distributed on the surface. The diameter of the pores is 1.13–1.66 μm and the distance between two pores is 2.90–4.77 μm. There are 2–5 granules on the operculum. The exine thickness is 0.43–1.77 μm. The number of microechini per sample area of 5 μm × 5 μm ranges from 9 to 14. The pollen grains have microechinate-perforate (Plate 17, figures 9–10), microechinate-punctate (Plate 17, figures 11–12) and microechinate-punctate-perforate ornamentation (Plate 17, figures 7–8).

3.9. *Schiedea*

Schiedea consists of 34 species worldwide (Bittrich 1993). In this research, we studied the species *Schiedea ligustrina* Cham. & Schlecht., and its pollen morphology is reported for the first time.

The pollen grains of *Schiedea* are spheroidal-polyhedral with a diameter of 17.14–20.56 μm, and have 14–18 round pores uniformly distributed on the surface. The diameter of the pores is 1.13–2.11 μm and the distance between two pores is 2.82–4.79 μm. There are 4–7 granules on the operculum. The exine thickness is 1.72–2.76 μm. The number of microechini per sample area of 5 μm × 5 μm ranges from 12

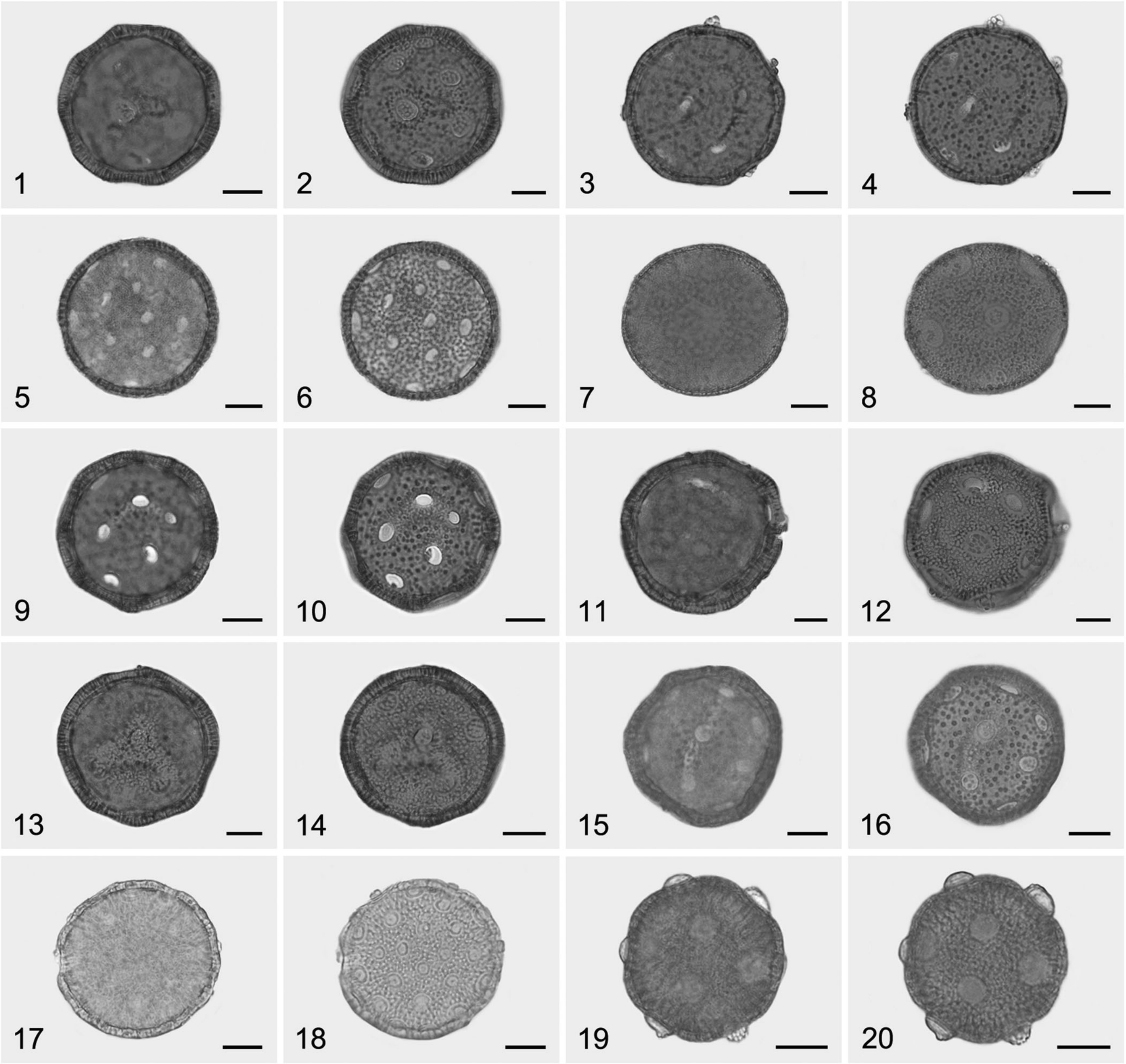

Plate 6. Light microscopy (LM) micrographs of tribe Alsineae pollen grains. 1–2. *Cerastium rubescens* var. *ovatum*; 3–4. *C. silvaticum*; 5–6. *C. takasagomontanum*; 7–8. *C. tianschanicum*; 9–10. *C. tomentosum*; 11–12. *C. trigynum* var. *morrisonen*; 13–14. *C. wilsoni*; 15–16. *Holosteum umbellatum*; 17–18. *Lepyrodiclis holosteoides*; 19–20. *Minuartia abchasica*. Scale bar: 10 μm.

to 20. The pollen grains have microechinate-punctate-perforate ornamentation (Plate 17, figures 13–14).

3.10. *Spergula*

Spergula consists of five species worldwide, with only one species in China (Lu et al. 2001). In this research, we studied the species *Spergula arvensis* L.

Pollen grains of *Spergula* are small in size. The average length of the polar axis is 18.06 (16.05–20.08) μm, and the average length of the equatorial axis is 15.87 (13.92–17.30) μm. The P/E ratio is 1.14, the grains being subprolate in shape. They are subcircular in equatorial view and semi-triangular in polar view, and tricolpate. The surface of the colpus membrane is granulate. The exine thickness is 0.75 (0.50–1.10) μm. The number of microechini per sample area of 5 μm × 5 μm ranges from 63 to 100. The pollen grains have microechinate-punctate-perforate ornamentation (Plate 17, figures 15–18).

3.11. *Spergularia*

Spergularia consists of 25 species worldwide, but there are only four species in China (Lu et al. 2001). We studied the species *Spergularia marina* (L.) Griseb.

Pollen grains of *Spergularia* are small in size. The average length of the polar axis is 28.40 (25.64–38.08) μm, and the

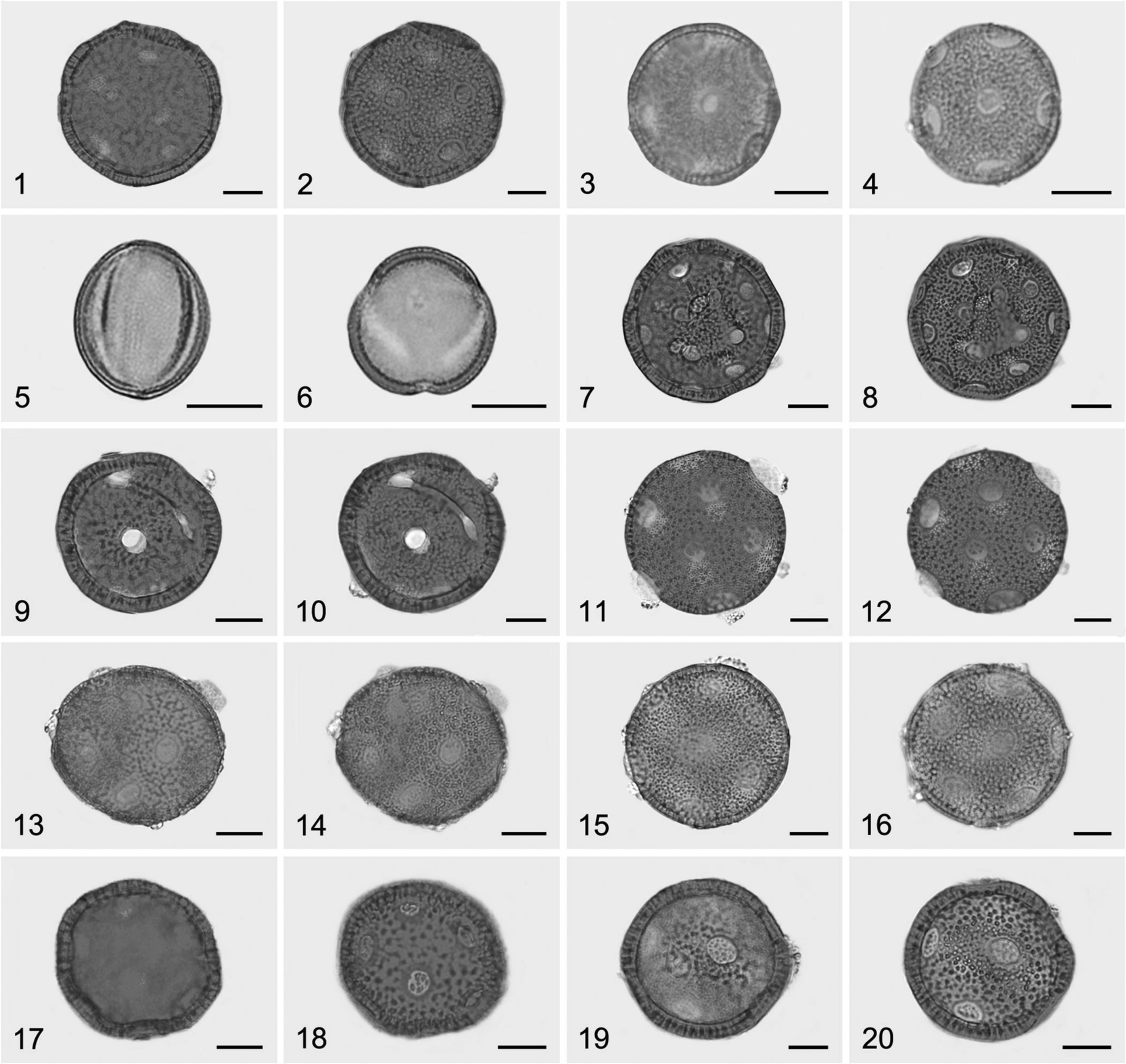

Plate 7. Light microscopy (LM) micrographs of tribe Alsineae pollen grains. 1–2. *Minuartia arctica* var. *hondoensis*; 3–4. *M. fasciculata*; 5–6. *M. geniculata*; 7–8. *M. hybrida*; 9–10. *M. kashmirica*; 11–12. *M. kitaibelii*; 13–14. *M. kryloviana*; 15–16. *M. laricifolia*; 17–18. *M. laricina*; 19–20. *M. lineata*. Scale bar: 10 μm.

average length of the equatorial axis is 16.95 (13.08–19.91) μm. The P/E ratio is 1.70, the grains being prolate in shape. They are subcircular in equatorial view and broad-elliptic in polar view, and tricolpate. The surface of the colpus membrane is microechinate. The exine thickness is 0.87 (0.64–1.39) μm. The number of microechini per sample area of 5 μm × 5 μm ranges from 65 to 100. The pollen grains have microechinate-punctate-perforate ornamentation (Plate 17, figures 18–20).

3.12. Statistical analysis

For quantitative analysis of the pollen grain size (excluding tricolpate pollen), we found that *Bufonia parviflora* is separated from the other species by a lower pollen diameter. *Arenaria trichophora* Franch. is separated from other species by a higher pollen diameter. The other species cannot be separated by pollen size because of the overlapping ranges of their pollen diameters (Figure 2).

4. Discussion

4.1. Pollen morphology

4.1.1. Pollen size

Our study revealed that the pollen of tribes Alsineae and Sperguleae was small (10–25 μm) or medium (26–50 μm) in size, consistent with the results of previous studies (Iwarsson 1977; Al-Eisawi 1989; Mostafavi and Mehregan 2014; Doğan and Ömer Erdem 2018). However, pollen size is affected by fixation media (such as glycerin gel, silicone oil, etc.), chemical treatment methods (acid treatment vs potassium hydroxide

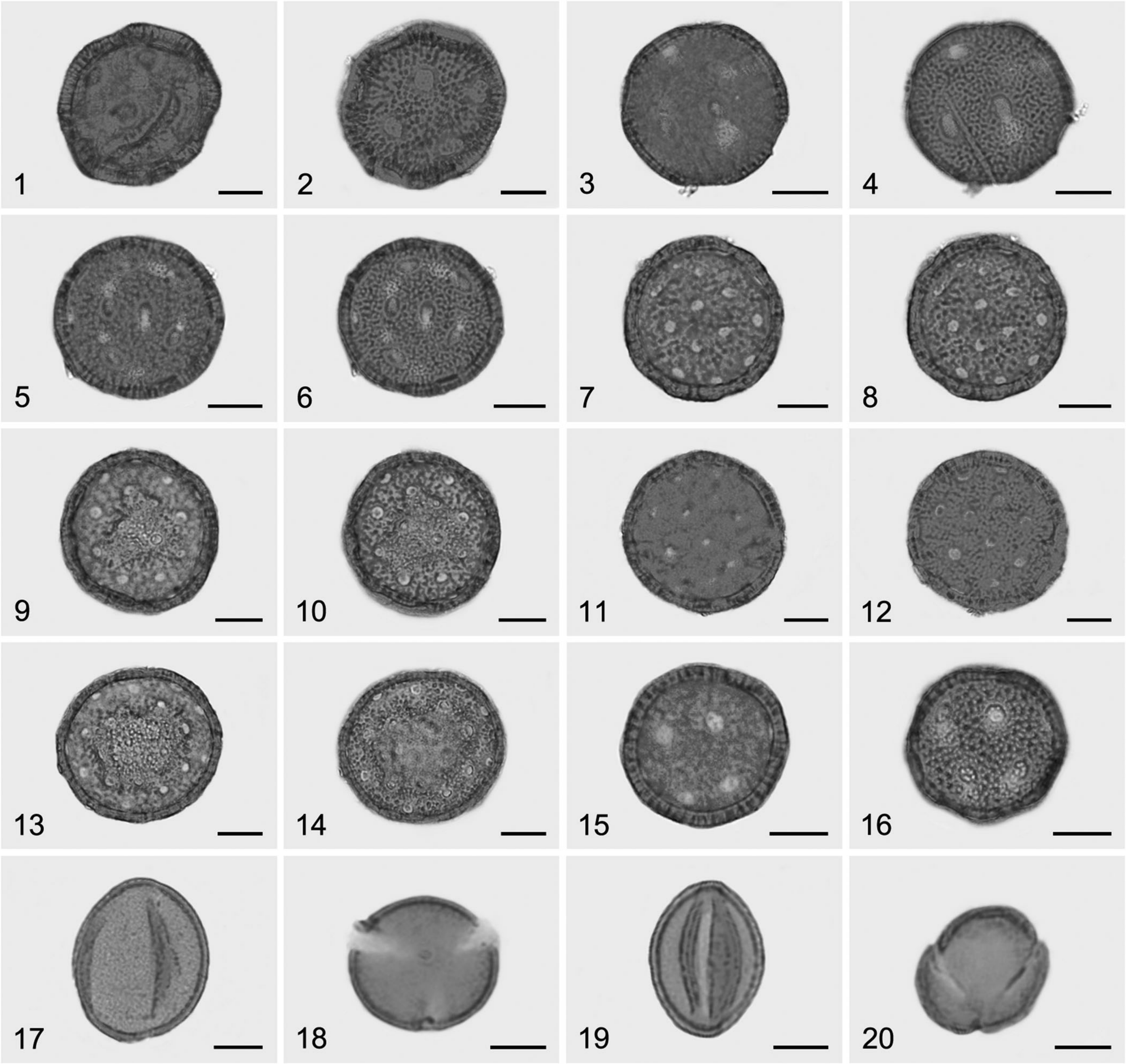

Plate 8. Light microscopy (LM) micrographs of tribes Alsineae and Sperguleae pollen grains. 1–2. *Minuartia macrocarpa* var. *koreana*; 3–4. *M. meyeri*; 5–6. *M. verna*; 7–8. *Sagina japonica*; 9–10. *S. maxima*; 11–12. *S. papuana*; 13–14. *S. saginoides*; 15–16. *Schiedea ligustrina*; 17–18. *Spergula arvensis*; 19–20. *Spergularia marina*. Scale bar: 10 μm.

treatment, dehydration vs non-dehydration, etc.) and some ecological factors (Punt and Hoen 1995; Torres 2000; Meltsov et al. 2008; Ataşlar et al. 2009; Wronska-Pilarek et al. 2010). Some scholars have suggested that pollen size is not meaningful in the taxonomy of Caryophyllaceae (Wan and Liu 2017; Cui et al. 2018; Wan et al. 2018). Our quantitative analysis showed that the pollen size of different species overlapped and thus could not be used to distinguish them (Figure 2).

4.1.2. Pollen shape

Our results show that the pollen grains of tribe Alsineae are spheroidal or spheroidal-polyhedral and the pollen grains of tribe Sperguleae are subprolate or prolate, consistent with previous results (Candau 1978; Perveen and Qaiser 2006; Chen et al. 2009). However, some studies have shown that pollen shape is susceptible to treatment methods (Moon et al. 2008; Fourny et al. 2018). In this study, the shape of pollen grains changed after acid treatment compared to that without treatment. For example, pollen grains of *Arenaria debilis* are spheroidal without acid treatment (Plate 1, figures 11–12). But after acid treatment, they become spheroidal-polyhedral (Plate 9, figures 11–12). By contrast, pollen grains of *Cerastium dahuricum* are spheroidal-polyhedral (Plate 12, figures 19–20), but after acid treatment, they become spheroidal (Plate 4, figures 11–12).

4.1.3. Number of pores

In this study, the number of pores of tribe Alsineae is 10–36, with the highest number in *Sagina*. Many studies have shown

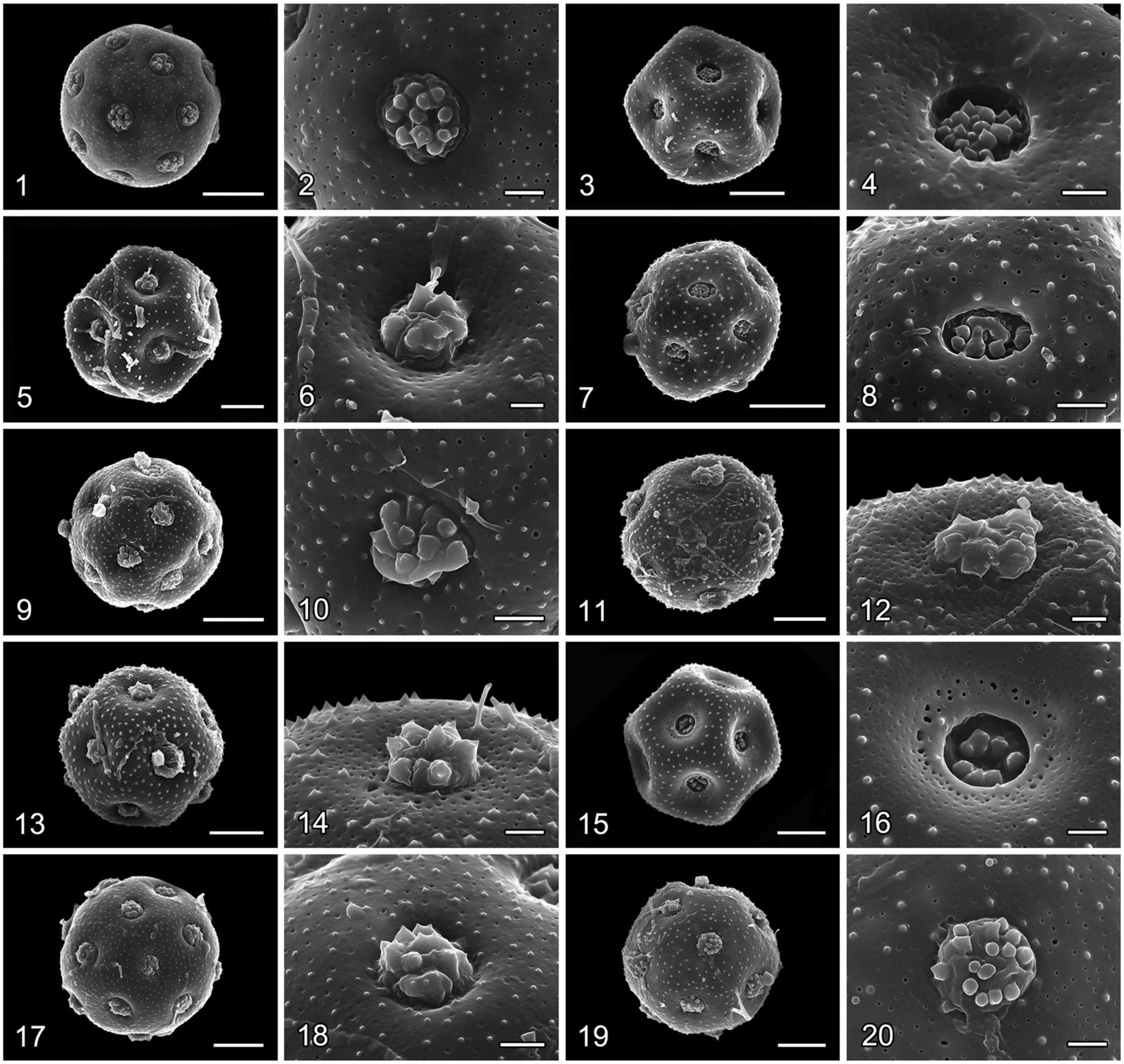

Plate 9. Scanning electron microscopy (SEM) micrographs of tribe Alsineae pollen grains. 1–2. *Arenaria arundana*; 3–4. *A. atuntziensis*; 5–6. *A. barbata*; 7–8. *A. brevipetala*; 9–10. *A. ciliata*; 11–12. *A. debilis*; 13–14. *A. delavayi*; 15–16. *A. dimorphitricha*; 17–18. *A. erinacea*; 19–20. *A. euodonta*. Scale bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 = 10 μm; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 = 2 μm.

that the number of pores is a stable trait and can be used as an important taxonomic basis at intergeneric, intrageneric and even interspecific levels (Faegri and Iversen 1975; Dang 1995; Yıldız 2001; Mostafavi and Mehregan 2014; Mačukanović-Jocić et al. 2015; Doğan and Ömer Erdem 2018; Ullah, Shah et al. 2019). Our results also show that the number of pores is relatively stable in each group, but varies among groups. Thus, it can be used as an important taxonomic index.

4.1.4. Density of microechini density

Many previous studies have suggested that the density of microechini on the surface of pollen in Caryophyllaceae can be used as an important basis for species classification (Aktaş et al. 2010; Mostafavi and Mehregan 2014; Ullah, Shah et al. 2019). Our results show that the pollen grains of all species have microechini on the surface. The density of microechini is relatively stable in each group but varies among groups (Tables S2–S4).

4.1.5. Species classification

The morphological characteristics of pollen grains are important for interspecific identification and can be used to distinguish species with similar external morphology (Mostafavi and Mehregan 2014; Wan and Liu 2017; Cui et al. 2018; Ullah, Zafar, Ahmad, Dilbar et al. 2018; Wan et al. 2018).

Arenaria longipetiolata is very similar to *A. yunnanensis* in terms of external morphology, except the shape of the leaf and petal tip (Tang et al. 1996). Therefore, these species are often misidentified if only macromorphological features are

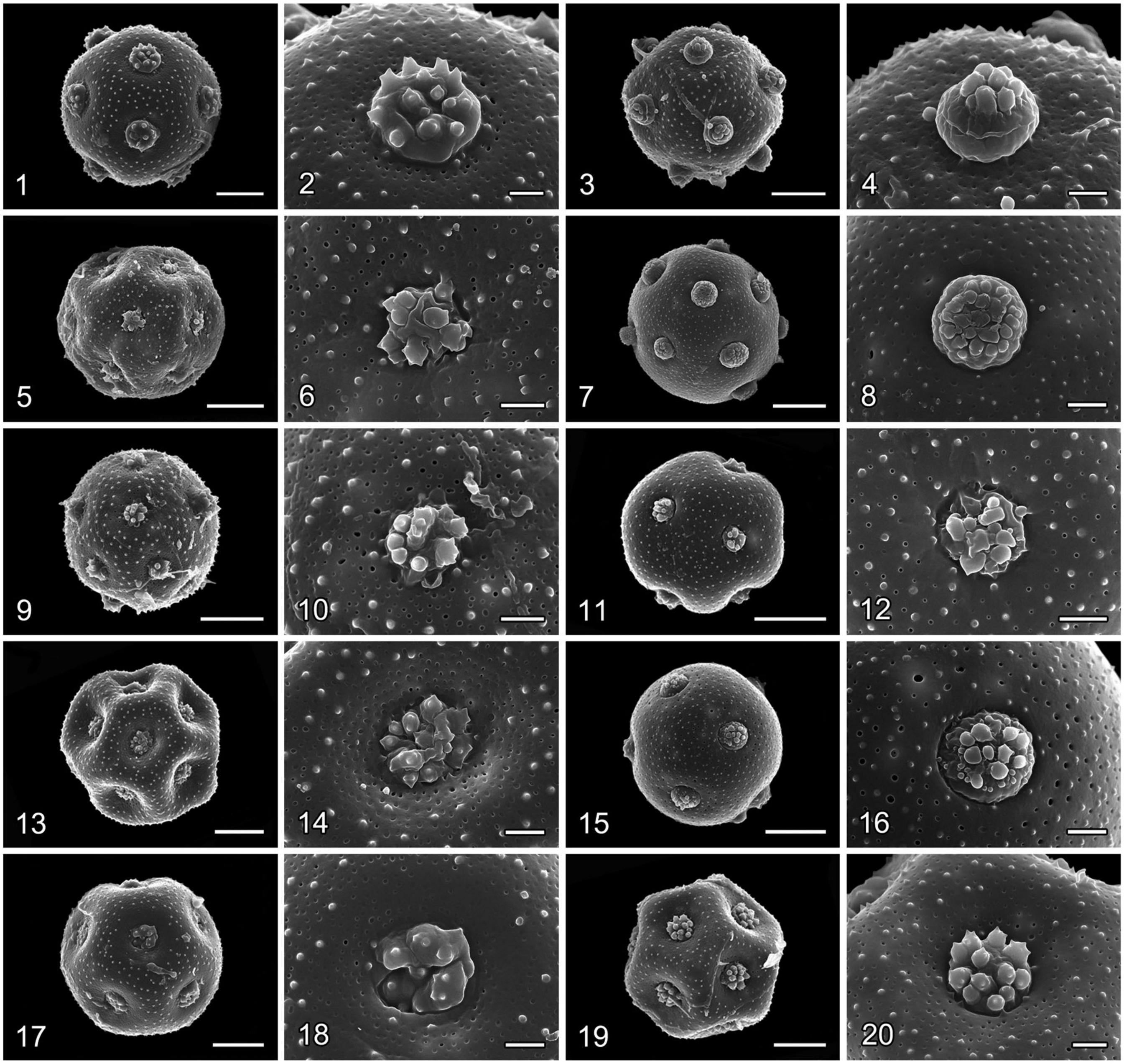

Plate 10. Scanning electron microscopy (SEM) micrographs of tribe Alsineae pollen grains. 1–2. *Arenaria fimbriata*; 3–4. *A. foliosa*; 5–6. *A. forrestii*; 7–8. *A. grandiflora*; 9–10. *A. ionandra*; 11–12. *A. juncea* var. *glabra*; 13–14. *A. leucasteria*; 15–16. *A. longifolia*; 17–18. *A. longipetiolata*; 19–20. *A. longistyla*. Scale bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 = 10 µm; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 = 2 µm.

examined. Our results show that their pollen grain diameter and exine thickness are similar, but the pore diameter and spacing of *A. longipetiolata* are smaller than those of *A. yunnanensis*. Although both have microechinate-punctate-perforate surface ornamentation (Plate 10, figures 17–18; Plate 12, figures 1–2), the density of microechini on the pollen surface of the former is higher than that of the latter. Therefore, these two species can be distinguished based on their pollen features.

Cerastium arvense differs from *C. arvense* var. *glabellum* only in whether the plant is pilose or glabrous (Tang et al. 1996). Our results show that although they have similar number of granules on the operculum, the pore diameter of the former is smaller than that of the latter, and the number of pores is 16 for the former and 13 for the latter. Although both have microechinate-punctate-perforate surface ornamentation (Plate 12, figures 11–14), the density of microechini on the pollen surface of the former is lower than that of the latter. Therefore, these two species can be distinguished based on their pollen features.

Cerastium fontanum differs from *C. fontanum* subsp. *vulgare* only in the length of the petal and sepal (Lu et al. 2001). Our results show that (i) they have similar pore diameter, exine thickness and number of granules on the operculum; (ii) the number of pores is 18 for the former and 14 for the latter; (iii) both have microechinate-punctate-perforate surface ornamentation (Plate 13, figures 3–6); (iv) the annulus of pollen grains of the former is smooth or barely microechinate, while that of the latter is microechinate. Therefore, pollen features can be used to distinguish the two species.

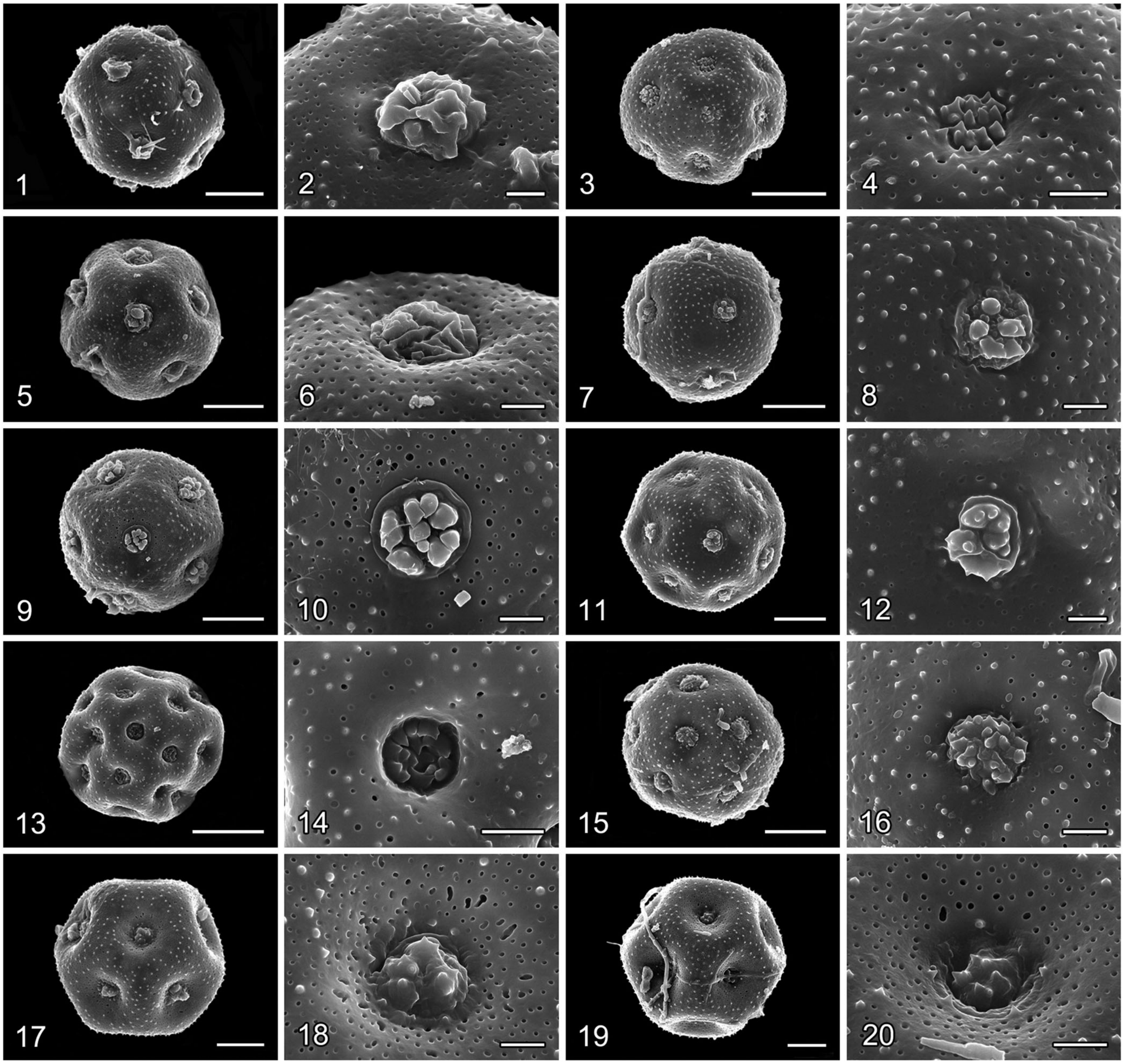

Plate 11. Scanning electron microscopy (SEM) micrographs of tribe Alsineae pollen grains. 1–2. *Arenaria napuligera*; 3–4. *A. orbiculata*; 5–6. *A. oreophila*; 7–8. *A. przewalskii*; 9–10. *A. pseudostellaria*; 11–12. *A. quadridentata*; 13–14. *A. serpyllifolia*; 15–16. *A. stenophylla*; 17–18. *A. trichophora*; 19–20. *A. xerophila*. Scale bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 = 10 μm; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 = 2 μm.

Cerastium fontanum subsp. *vulgare* and *C. glomeratum* are often confused in external morphology, with only subtle differences in their leaf shape and inflorescence (Tang et al. 1996). Our results show that although their pollen diameter, pore number and exine thickness are similar, their surface ornamentation is obviously different. The former has microechinate-punctate-perforate surface ornamentation (Plate 13, figures 5–6), while the latter has microechinate-punctate surface ornamentation (Plate 13, figures 9–10). Therefore, their pollen characteristics set them apart.

The external morphology of *Cerastium glutinosum* is very similar to that of *C. pumilum*, but can be distinguished by whether their bracts have scarious edges. Unfortunately, the morphology of the bracts has a transitional state (Smejkal 1990). Because the two are often confused in terms of external morphology, Clapham (1962) merged them into one species, *C. pumilum*. However, many studies have also identified *C. glutinosum* as a subspecies of *C. pumilum* (Graebner 1919; Prodan 1953; Jalas et al. 1964; Sell and Whitehead 1964; Soó 1970) or a variety of *C. pumilum* (Kulczyński 1921; Rico 1990). Our results show that the pollen grains of the two species are very similar and do not differ significantly in terms of pollen diameter, number of pores, number of granules on the operculum or density of microechini on the pollen surface. In addition, both have microechinate-punctate-perforate surface ornamentation (Plate 13, figures 11–12; Plate 14, figures 13–14). These results support the decision of Clapham (1962) to combine them into one species.

Minuartia lineata and *M. kashmirica* both belong to the section *Acutiflorae*. Some studies have merged them into a

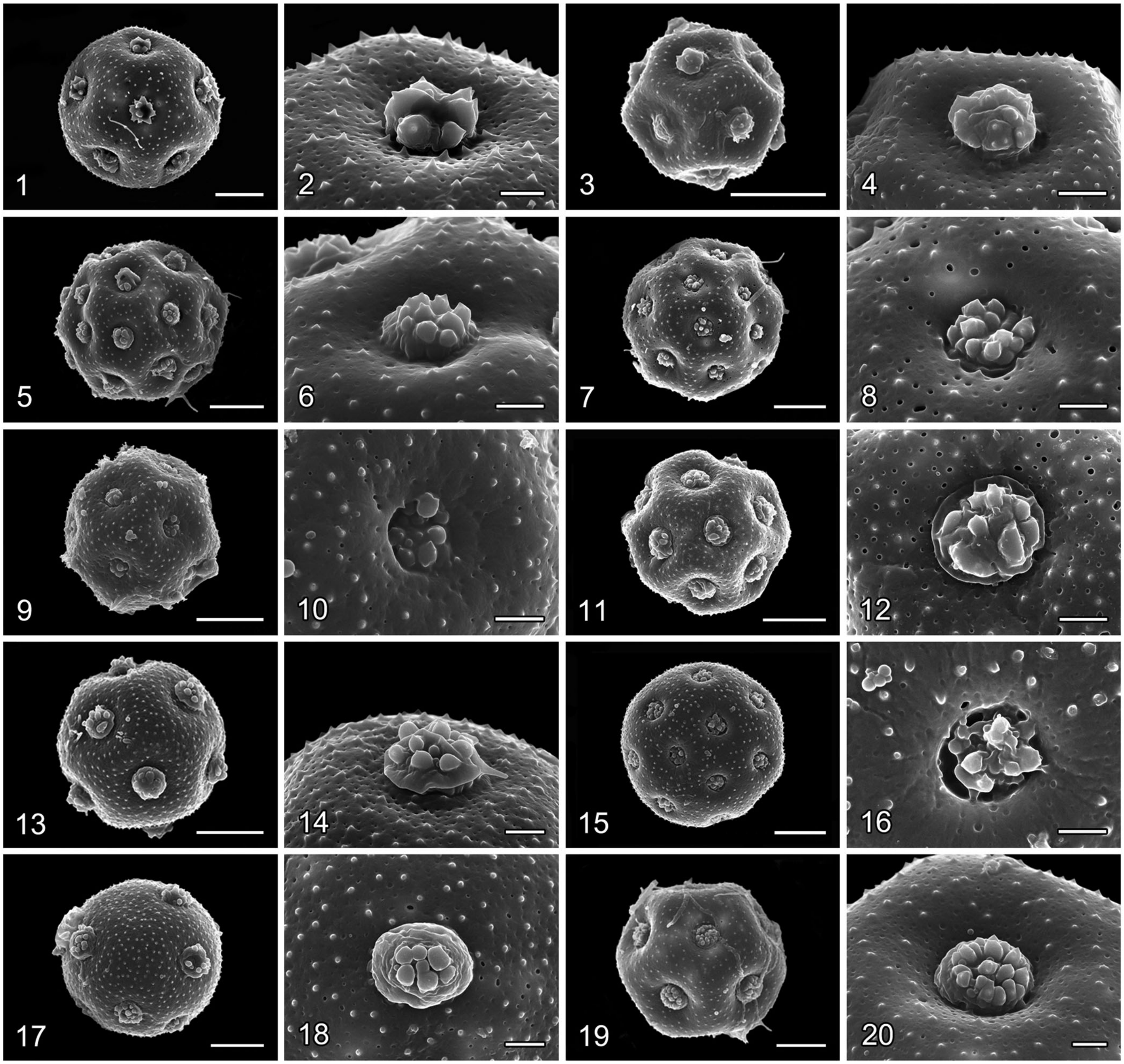

Plate 12. Scanning electron microscopy (SEM) micrographs of tribe Alsineae pollen grains. 1–2. *Arenaria yunnanensis*; 3–4. *Bufonia parviflora*; 5–6. *Cerastium alpinum*; 7–8. *C. alsinifolium*; 9–10. *C. anomalum*; 11–12. *C. arvense*; 13–14. *C. arvense* var. *glabellum*; 15–16. *C. baischanense*; 17–18. *C. cerastoides*; 19–20. *C. dahuricum*. Scale bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 = 10 μm; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 = 2 μm.

single species, namely *M. kashmirica* (Ghazanfar and Nasir 1986), while others suggested that they should be independent species (McNeill 1962; Marhod 2011). Our results show that the two species have similar exine thickness, but the former has a smaller pore diameter than the latter. In addition, the former has microechinate-punctate surface ornamentation (Plate 16, figures 15–16), while the latter has microechinate-punctate-perforate surface ornamentation (Plate 16, figures 5–6). Therefore, the pollen grain features support the independent existence of the two species.

4.2. Infrageneric classification of Arenaria

Since the establishment of *Arenaria*, its scope has been controversial (Fenzl 1840, 1842; Bentham and Hooker 1862; Williams 1895; Maguire 1951). Don (1831) separated the subgenus *Odontostemma* from *Arenaria* and listed it as a genus. However, some studies still regard it as *Arenaria* subgenus *Odontostemma* (Williams 1898; McNeill 1962; Wu et al. 2001; Rabeler and Hartman 2005).

Recent studies of phylogenetic relationships indicate that *Arenaria* is not a monophyletic group and subgenus *Odontostemma* could be clustered into a monophyletic group, which would support restoring the subgenus *Odontostemma* to *Odontostemma* (Fior et al. 2006; Harbaugh et al. 2010; Greenberg and Donoghue 2011; Dillenberger and Kadereit 2014; Sadeghian et al. 2015). This view has gained the support of many scholars (Hernández-Ledesma et al. 2015; Richard and Wagner 2016; Yao 2017).

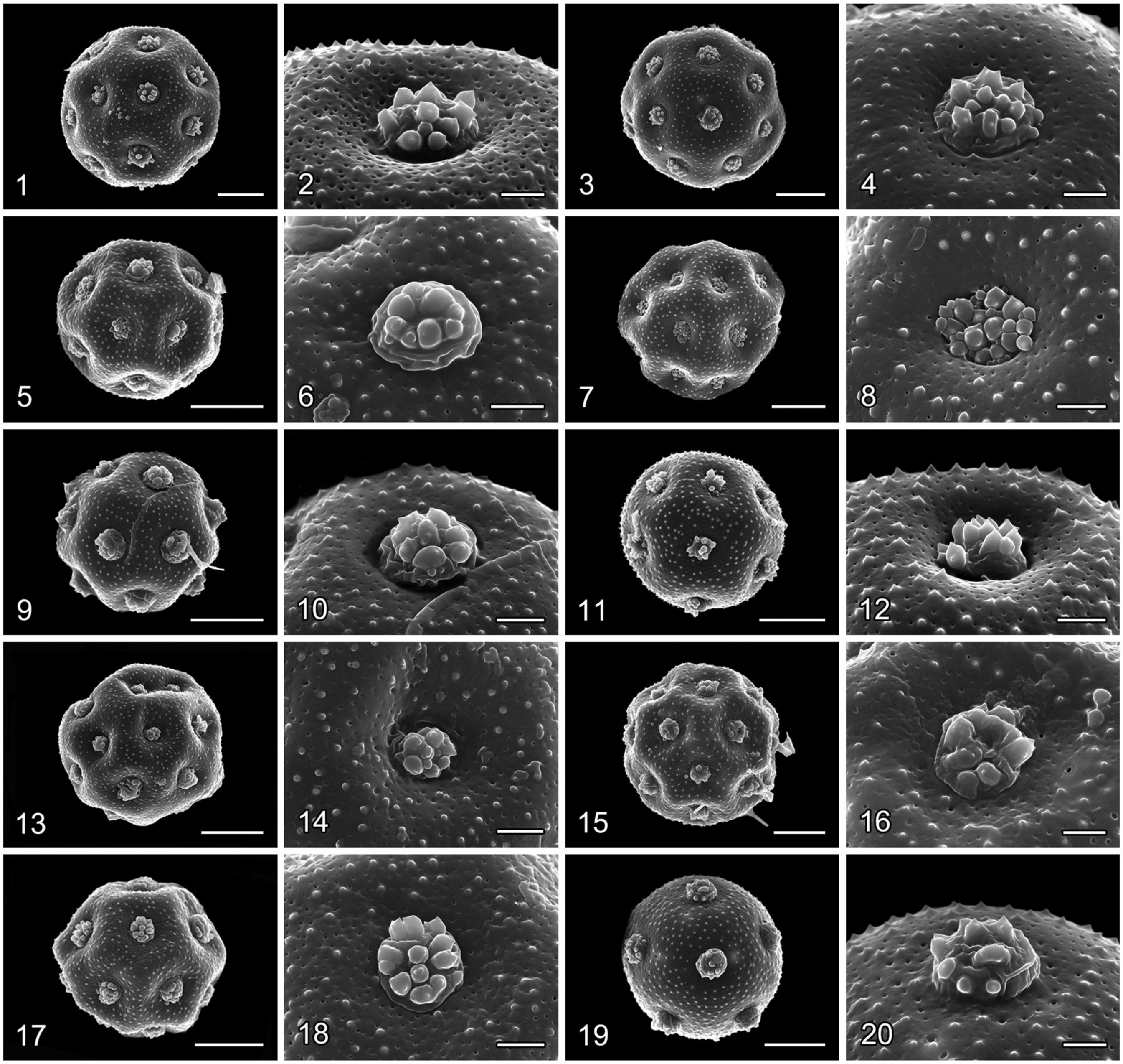

Plate 13. Scanning electron microscopy (SEM) micrographs of tribe Alsineae pollen grains. 1–2. *Cerastium fischerianum*; 3–4. *C. fontanum*; 5–6. *C. fontanum* subsp. *vulgare*; 7–8. *C. furcatum*; 9–10. *C. glomeratum*; 11–12. *C. glutinosum*; 13–14. *C. holosteoides* var. *angustifolium*; 15–16. *C. limprichtii*; 17–18. *C. lithospermifolium*; 19–20. *C. maximum*. Scale bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 = 10 µm; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 = 2 µm.

Our results show that the diameter of the pollen grains, diameter and number of pores, density of microechini, and surface ornamentation of subgenus *Odontostemma* are uniform. Therefore, pollen characteristics support subgenus *Odontostemma* as a monophyletic group.

For subgenus *Odontostemma*, the number of microechini in a 5 µm × 5 µm area of pollen grain surface is 7–10 (fewer than 11); the annulus of pollen grains is smooth or barely microechinate; and the surface ornamentation of pollen grains is microechinate-punctate-perforate. For the other species of *Arenaria*, the number of microechini in a 5 µm × 5 µm area of the pollen grain surface is 11–15 (all greater than 10); the annulus of the pollen grains is microechinate; and the surface ornamentation is microechinate-perforate, microechinate-punctate, or microechinate-punctate-perforate. Therefore, pollen characteristics support distinguishing subgenus *Odontostemma* from *Arenaria*, and making it an independent genus.

4.3. Systematic position of subgenus Dichodon

Since the establishment of *Dichodon* by Reichenbach (1841), its systematic position has been controversial. Schischkin (1970) considered *Dichodon* as a subgenus of *Cerastium* based on morphological characteristics. However, some studies suggest that subgenus *Dichodon* should be separated from *Cerastium* and classified as *Dichodon* (Sokolova 1996; Hernández-Ledesma et al. 2015; Yao 2016).

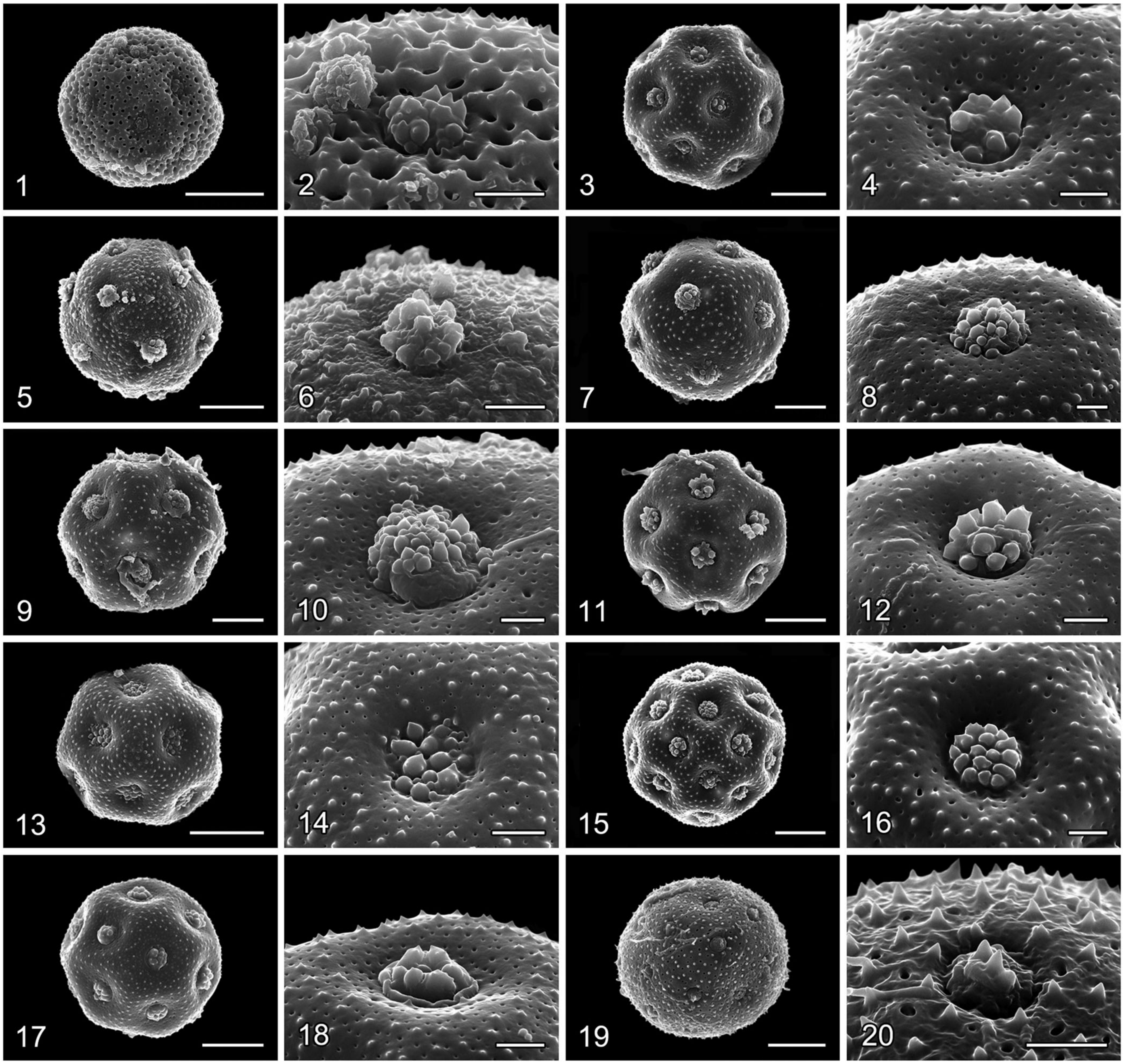

Plate 14. Scanning electron microscopy (SEM) micrographs of tribe Alsineae pollen grains. 1–2. *Cerastium nutans*; 3–4. *C. oblongifolium*; 5–6. *C. papuanum*; 7–8. *C. pauciflorum* var. *oxalidiflorum*; 9–10. *C. polymorphum*; 11–12. *C. ponticum*; 13–14. *C. pumilum*; 15–16. *C. rubescens* var. *ovatum*; 17–18. *C. silvaticum*; 19–20. *C. takasagomontanum*. Scale bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 = 10 μm; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 = 2 μm.

Arabi et al. (2017) found that subgenus *Dichodon* and other species of *Cerastium* have significant differences in seed colour and seed surface ornamentation, supporting the resurrection of *Dichodon* at the generic level.

All the latest molecular phylogenetic analysis indicates that subgenus *Dichodon* is a monophyletic group. In the phylogenetic tree, subgenus *Dichodon* does not form a sister group with other species of *Cerastium*, but forms a sister group with *Holosteum* (Arabi et al. 2018). These results support separating the subgenus *Dichodon* from *Cerastium* and raising it to generic rank.

Our study found that pollen grains of subgenus *Dichodon* have 10–11 pores (all less than 12), and microechinate-punctate-perforate surface ornamentation. By contrast, the pollen grains of the other species of *Cerastium* have 12–20 pores (mostly 14–20; all more than 11). Besides microechinate-punctate-perforate surface ornamentation, the pollen grains of other species of *Cerastium* have microechinate-perforate and microechinate-punctate surface ornamentations. Therefore, the pollen features support the separation of subgenus *Dichodon* from the genus *Cerastium* and the classification of *Dichodon* as an independent genus.

4.4. Infrageneric classification of *Minuartia*

Mattfeld (1922) carried out an infrageneric classification of *Minuartia* based on morphological features. Later, McNeill

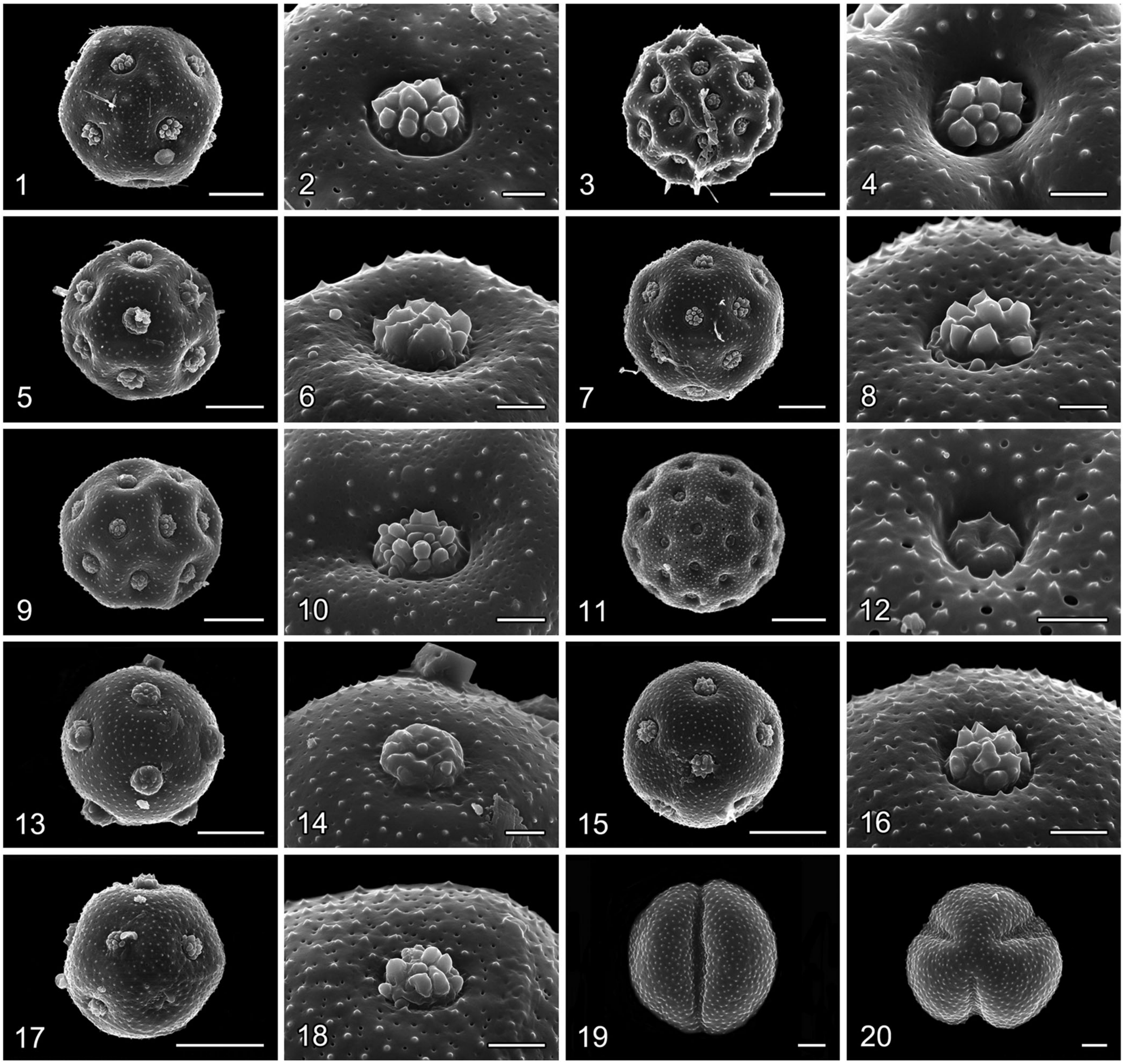

Plate 15. Scanning electron microscopy (SEM) micrographs of tribe Alsineae pollen grains. 1–2. *Cerastium tianschanicum*; 3–4. *C. tomentosum*; 5–6. *C. trigynum* var. *morrisonen*; 7–8. *C. wilsoni*; 9–10. *Holosteum umbellatum*; 11–12. *Lepyrodiclis holosteoides*; 13–14. *Minuartia abchasica*; 15–16. *M. arctica* var. *hondoensis*; 17–18. *M. fasciculata*; 19–20. *M. geniculata*. Scale bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 20 = 10 µm; 2, 4, 6, 8, 10, 12, 14, 16, 18 = 2 µm.

(1962) revised the classification and divided *Minuartia* into four subgenera, of which subgenus *Minuartia* is the largest and is further divided into 12 sections.

However, phylogenetic relationships revealed in recent years indicate that *Minuartia* is not a monophyletic group (Fior et al. 2006; Harbaugh et al. 2010; Greenberg and Donoghue 2011), and some sections in the subgenus *Minuartia* have also been proven not to be monophyletic. For example, section *Spectabiles* is embedded in different clades in the phylogenetic tree (Dillenberger and Kadereit 2014).

The study found that the pollen diameter, aperture type and surface ornamentations of *Minuartia* vary greatly. Therefore, pollen characteristics support the claim that *Minuartia* is not a monophyletic group.

Minuartia arctica var. *hondoensis*, *M. kitaibelii*, *M. laricifolia*, *M. laricina*, and *M. macrocarpa* var. *koreana* in this study all belong to section *Spectabiles* (McNeill 1962). A comparison of their pollen grain morphological characteristics showed that surface ornamentation, number of pores and density of microechini of section *Spectabiles* vary greatly. Therefore, pollen characteristics support the idea that the section *Spectabiles* is not a monophyletic group.

In the phylogenetic tree of Dillenberger and Kadereit (2014), section *Sabulina* of subgenus *Minuartia* forms a clade with section *Tryphane*, and section *Acutiflorae* forms another clade; these two clades are sister groups. *Minuartia hybrida* and *M. kryloviana* both belong to section *Sabulina*, while *M. verna* belongs to section *Tryphane*, and *M. kashmirica* and

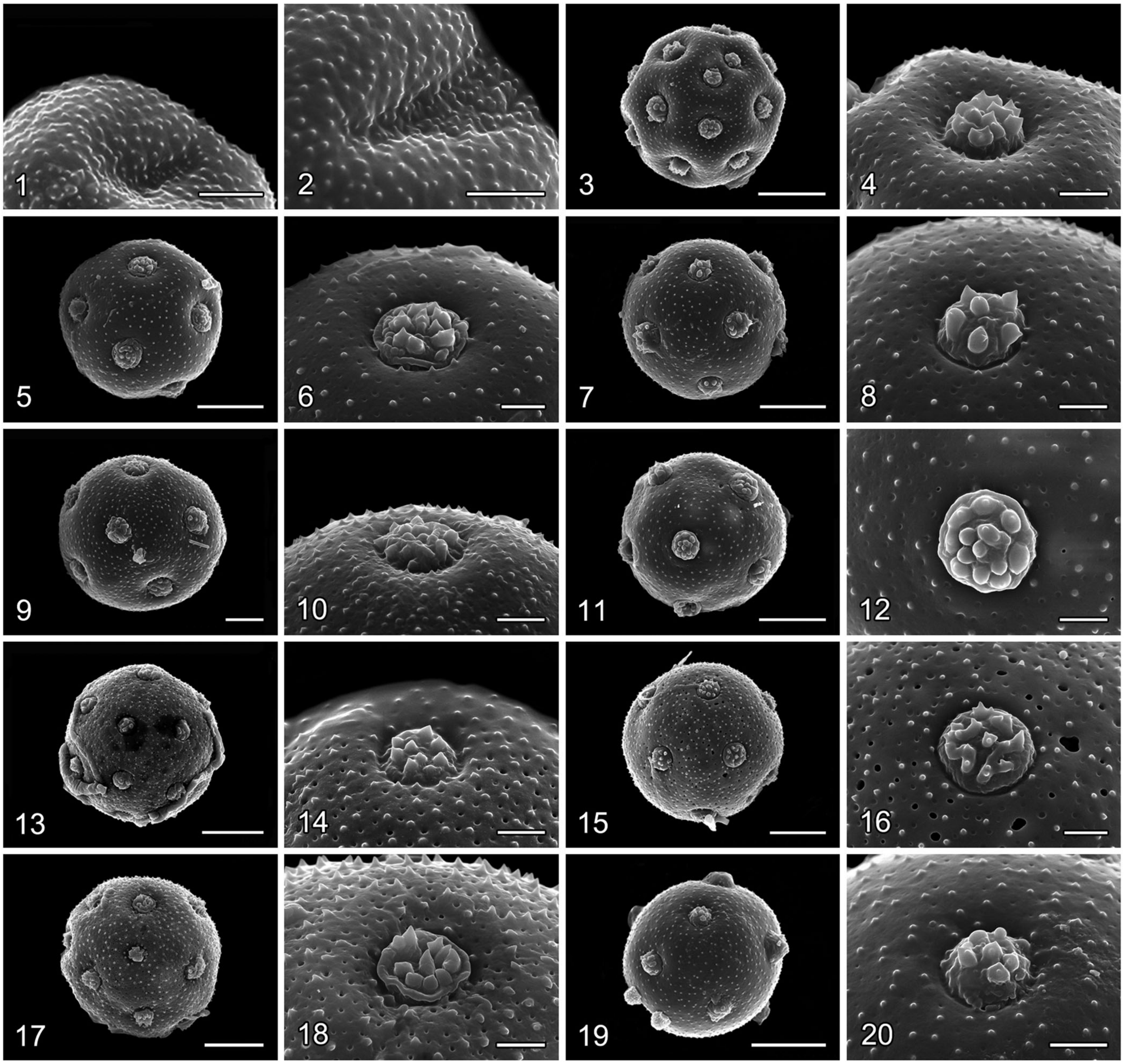

Plate 16. Scanning electron microscopy (SEM) micrographs of tribe Alsineae pollen grains. 1–2. *Minuartia geniculata*; 3–4. *M. hybrida*; 5–6. *M. kashmirica*; 7–8. *M. kitaibelii*; 9–10. *M. kryloviana*; 11–12. *M. laricifolia*; 13–14. *M. laricina*; 15–16. *M. lineata*; 17–18. *M. macrocarpa* var. *koreana*; 19–20. *M. meyeri*. Scale bars: 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 = 2 μm; 3, 5, 7, 9, 11, 13, 15, 17, 19 = 10 μm.

M. lineata belong to section *Acutiflorae* (McNeill 1962). Comparing the morphological characteristics of pollen grains shows that the two clades have similar pollen diameter, pore diameter and distance between two pores, and the same surface ornamentation. Therefore, pollen features support recognizing the two clades as sister groups.

4.5. Phylogenetic position of subgenus Rhodalsine

Gay (1845) established *Rhodalsine*, which included only *Rhodalsine procumbens*, and Ascherson and Graebner (1918) argued that *Rhodalsine* should be a subgenus of *Minuartia* (*Minuartia* subgenus *Rhodalsine*). Later, some authors classified it into tribe Alsineae (McNeill 1962; Bittrich 1993). However, there are still some authors arguing that *Rhodalsine* should be separated from *Minuartia* and listed as a genus (Phitos 1997; Kool and Thulin 2013, 2017b).

McNeill and Basset (1974) compared the characteristics of petals and seeds and the number of chromosomes of *Minuartia* and *Spergularia*, and found that subgenus *Rhodalsine* varied greatly in morphological features from *Minuartia*. It has the same number of chromosomes as and similar pink petals to *Spergularia*, but significantly different seed characteristics. Therefore, whether *Rhodalsine* should be classified into tribe Sperguleae is still unclear.

Recent phylogenetic studies have shown that the subgenus *Rhodalsine* is not closely related to other species of *Minuartia* and is clustered together with the tribe Sperguleae, thus supporting the restoration of subgenus

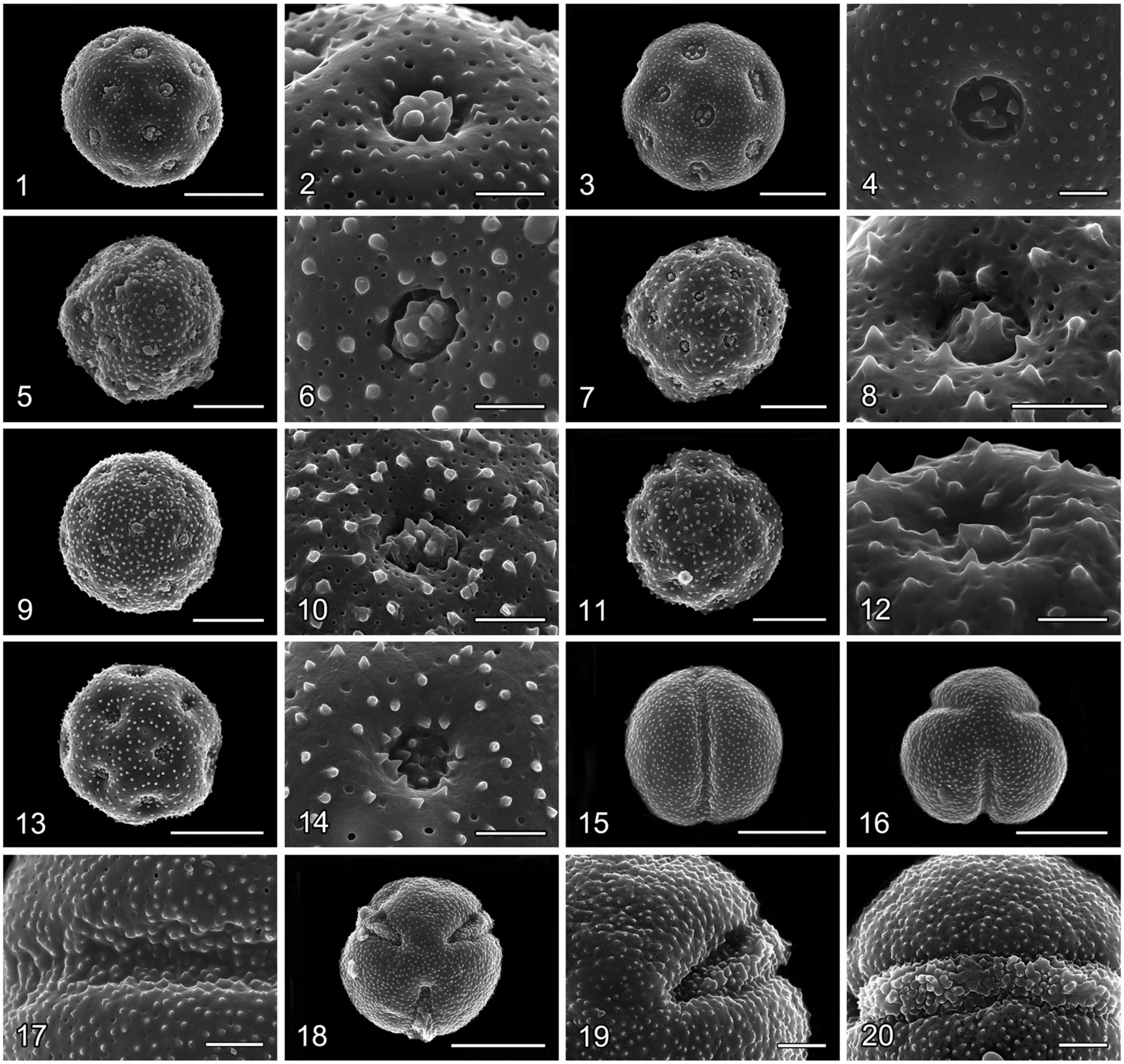

Plate 17. Scanning electron microscopy (SEM) micrographs of tribes Alsineae and Spergueae pollen grains. 1–2. *Minuartia verna*; 3–4. *Moehringia trinervia*; 5–6. *Sagina japonica*; 7–8. *S. maxima*; 9–10. *S. papuana*; 11–12. *S. saginoides*; 13–14. *Schiedea ligustrina*; 15–17. *Spergula arvensis*; 18–20. *Spergularia marina*. Scale bars: 1, 3, 5, 7, 9, 11, 13, 15, 16, 18 = 10 μm; 2, 4, 6, 8, 10, 12, 14, 17, 19, 20 = 2 μm.

Rhodalsine to *Rhodalsine*, and further classifying it in the tribe Sperguleae, but not the tribe Alsineae (Harbaugh et al. 2010; Greenberg and Donoghue 2011; Dillenberger and Kadereit 2014; Kool and Thulin 2017a).

Our results show that the subgenus *Rhodalsine* has tricolpate pollen and microechinate-punctate surface ornamentation, and a granulate colpus membrane surface (Plate 15, figures 19–20; Plate 16, figures 1–2). However, other species of *Minuartia* have pantoporate pollen. Besides microechinate-punctate surface ornamentation, the pollen grains of other species of *Minuartia* display microechinate-perforate and microechinate-punctate-perforate ornamentation. Therefore, pollen characteristics support the separation of subgenus *Rhodalsine* from *Minuartia* as an independent genus.

In addition, *Rhodalsine* and the tribe Sperguleae have similar pollen grains with tricolpate apertures, while other species of the tribe Alsineae have pantoporate apertures. Taia (1994) found that the pollen grains of *Rhodalsine* are similar to those of *Spergularia*, although *Spergularia* has a granulate colpus membrane surface. They believed that *Rhodalsine* should be classified into tribe Sperguleae. However, Taia (1994) did not describe the pollen characteristics of *Rhodalsine* and *Spergularia* in detail. Due to the low resolution of the images, the pollen characteristics of *Rhodalsine* and *Spergularia* are hardly observed. Our results show that the pollen characteristics of *Rhodalsine* and *Spergula* are more similar. Both have a granulate colpus membrane surface and sunken colpus membrane (Plate 16, figures 1–2; Plate 17, figures 15–17), while *Spergularia* has a microechinate colpus membrane surface and protrusive colpus

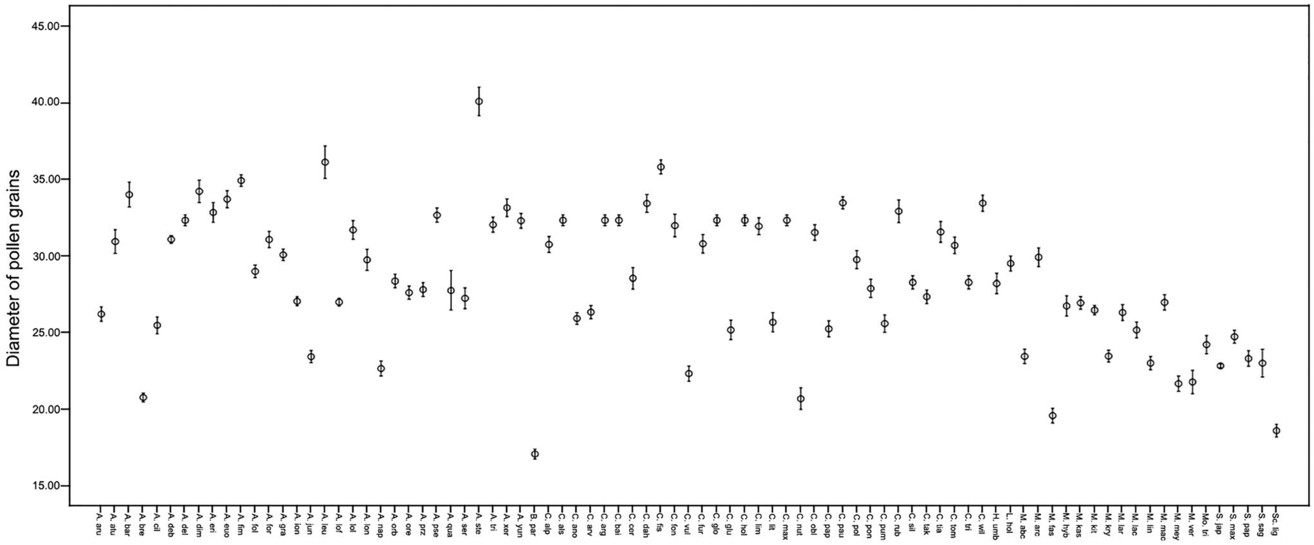

Figure 2. Representation of the confidence interval of the mean in 95% of the diameter of pollen grains. The x-axis represents the analysed species of tribes Alsineae and Sperguleae and the y-axis represents the diameter of pollen grains. The higher and lower boundaries show the confidence interval; the average circle shows the arithmetic mean. A. aru = *Arenaria arundana*; A. atu = *A. atuntziensis*; A. bar = *A. barbata*; A. bre = *A. brevipetala*; A. cil = *A. ciliata*; A. deb = *A. debilis*; A. del = *A. delavayi*; A. dim = *A. dimorphitricha*; A. eri = *A. erinacea*; A. euo = *A. euodonta*; A. fim = *A. fimbriata*; A. fol = *A. foliosa*; A. for = *A. forrestii*; A. gra = *A. grandiflora*; A. ion = *A. ionandra*; A. jun = *A. juncea* var. *glabra*; A. leu = *A. leucasteria*; A. lof = *A. longifolia*; A. lol = *A. longipetiolata*; A. lon = *A. longistyla*; A. nap = *A. napuligera*; A. orb = *A. orbiculata*; A. ore = *A. oreophila*; A. prz = *A. przewalskii*; A. pse = *A. pseudostellaria*; A. qua = *A. quadridentata*; A. ser = *A. serpyllifolia*; A. ste = *A. stenophylla*; A. tri = *A. trichophora*; A. xer = *A. xerophila*; A. yun = *A. yunnanensis*; B. par = *Bufonia parviflora*; C. alp = *Cerastium alpinum*; C. als = *C. alsinifolium*; C. ano = *C. anomalum*; C. arv = *C. arvense*; C. arg = *C. arvense* var. *glabellum*; C. bai = *C. baischanense*; C. cer = *C. cerastoides*; C. dah = *C. dahuricum*; C. fis = *C. fischerianum*; C. fon = *C. fontanum*; C. vul = *C. fontanum* subsp. *vulgare*; C. fur = *C. furcatum*; C. glo = *C. glomeratum*; C. glu = *C. glutinosum*; C. hol = *C. holosteoides* var. *angustifolium*; C. lim = *C. limprichtii*; C. lim = *C. lithospermifolium*; C. max = *C. maximum*; C. nut = *C. nutans*; C. nut = *C. oblongifolium*; C. pap = *C. papuanum*; C. pau = *C. pauciflorum* var. *oxalidiflorum*; C. pol = *C. polymorphum*; C. pon = *C. ponticum*; C. pum = *C. pumilum*; C. rub = *C. rubescens* var. *ovatum*; C. sil = *C. silvaticum*; C. tak = *C. takasagomontanum*; C. tia = *C. tianschanicum*; C. tom = *C. tomentosum*; C. tri = *C. trigynum* var. *morrisonen*; C. wil = *C. wilsoni*; H. umb = *Holosteum umbellatum*; L. hol = *Lepyrodiclis holosteoides*; M. abc = *Minuartia abchasica*; M. arc = *M. arctica* var. *hondoensis*; M. fas = *M. fasciculata*; M. hyb = *M. hybrida*; M. kas = *M. kashmirica*; M. kas = *M. kitaibelii*; M. kry = *M. kryloviana*; M. lar = *M. laricifolia*; M. lac = *M. laricina*; M. lin = *M. lineata*; M. mac = *M. macrocarpa* var. *koreana*; M. mey = *M. meyeri*; M. ver = *M. verna*; Mo. tri = *Moehringia trinervia*; S. jap = *Sagina japonica*; S. max = *S. maxima*; S. pap = *S. papuana*; S. sag = *S. saginoides*; Sc. lig = *Schiedea ligustrina*.

membrane (Plate 17, figures 18–20). Therefore, pollen characteristics support classifying *Rhodalsine* in the tribe Sperguleae, but within the tribe Alsineae, and suggest it is closer to *Spergula*.

4.6. Classification of tribe Alsineae

4.6.1. Relationship between *Sagina* and tribe Alsineae

The latest phylogenetic studies suggest that *Sagina* should be separated from tribe Alsineae (Harbaugh et al. 2010; Greenberg and Donoghue 2011). Based on the characteristics of the seeds, Arabi et al. (2017) found that the seed size and the cellular characteristics of exotesta of *Sagina* were significantly different from those of other species of tribe Alsineae.

Our results show that for *Sagina*, the pore diameter is 1.13–1.66 μm, the number of granules on the operculum is 2–5, the number of pores is 24–36, and the surface ornamentation is microechinate-punctate, microechinate-perforate or microechinate-punctate-perforate. In comparison, in other species of tribe Alsineae the pore diameter is 1.72–5.60 μm and the number of granules on the operculum is 3–13; for many genera, the number of pores is no more than 20 (only for *Lepyrodiclis* is the number of pores 28–32). In addition, microechinae on the pollen surface of *Sagina* are larger than those on the pollen surface of other species of tribe Alsineae. These results are consistent with those of Punt and Hoen (1995). Therefore, the pollen characteristics support the separation of *Sagina* from tribe Alsineae.

4.6.2. The relationship among subgenus *Eremogone*, subgenus *Eremogoneastrum* and tribe Alsineae

Harbaugh et al. (2010) conducted a phylogenetic study and found that subgenus *Eremogone* and subgenus *Eremogoneastrum* are clustered together and have a close relationship with tribe Caryophylleae. Therefore, they believed that these two subgenera should be separated from the tribe Alsineae. Greenberg and Donoghue (2011) expanded the sample size of the research and supported the view of Harbaugh et al. (2010).

Our results show that the pollen diameter, pore diameter and distance between two pores, number of granules on the operculum, number of pores, density of microechini and surface ornamentation of subgenus *Eremogone* and subgenus *Eremogoneastrum* all conform to the characteristics of tribe Alsineae. These pollen characteristics do not support the molecular phylogenetic views. But because of the small sample size of subgenus *Eremogone* and subgenus *Eremogoneastrum* in this study, their relationship with the tribe Alsineae needs to be studied further.

4.7. The relationship between tribe Alsineae and tribe Sperguleae

Van Campo (1967) believed that the aperture type of pollen grains evolves from the colpate type to the porate type. Iwarsson (1977) pointed out that pantoporate pollen grains are more advanced when studying the pollen of Caryophyllaceae.

Dang and Jiang (1996) also compared the pollen grains of the subfamily Paronychioideae, and believed that the porate group was more derived while the colpate group are more primitive.

Our results also show that members of the tribe Alsineae have pantoporate pollen while members of the tribe Sperguleae have tricolpate pollen. Therefore, according to pollen characteristics, tribe Alsineae is more evolved than tribe Sperguleae.

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