ABS ACTA BIOLOGICA SIBIRICA
A journal on the biodiversity of Siberia and the adjacent lands

Acta Biologica Sibirica 11: 773–797 (2025)
doi: 10.5281/zenodo.16563435
http://journal.asu.ru

RESEARCH ARTICLE

New data on the morphology of pollen of *Pulsatilla* Mill. (Ranunculaceae)

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Academic editor: R. Yakovlev | Received 12 June 2025 | Accepted 10 July 2025 | Published 31 July 2025

http://zoobank.org/1AB156CF-7976-4438-8FBB-8445172C0693

Citation: Zaikov VF, Shalimov AP, Vaganov AV, Sinitsyna TA, Kudrin SG, Kechaykin AA, Ryabova KK, Pankratov SYu, Batkin AA, Shmakov AI (2025) New data on the morphology of pollen of *Pulsatilla* Mill. (Ranunculaceae). Acta Biologica Sibirica 11: 773–797. https://doi.org/10.5281/zenodo.16563435

Abstract

The genus *Pulsatilla* Mill. represents a taxonomically complex group, with its systematic classification having undergone numerous revisions. Pollen grain morphology plays a crucial role in the intrageneric taxonomy of *Pulsatilla*; however, it remains unexamined for several species within the genus. This study presents the results of a comprehensive palynological analysis of 18 *Pulsatilla* species, including nine species investigated for the first time. The majority of species exhibited a 3-colpate aperture, including *P. astragalifolia*, *P. bungeana*, *P. ajanensis*, *P. cernua*, *P. ambigua*, *P. taurica*, *P. kryloviana*, *P. herba-somnii*, *P. turczaninovii*, *P. multifida*, and *P. archarensis*. A pantocolpate aperture was characteristic of *P. herbertii* and *P. neobungeana*, while *P. campanella*, *P. andina*, and *P. neobungeana* displayed a pantoporate aperture. The exine sculpture of all examined pasqueflowers was consistently microspinate, with predominantly smooth surfaces, except for occasional tuberculate textures. Pollen grain shapes varied from elongated ellipsoidal to spheroidal forms.

Keywords
Herbarium, morphology, palynology, pollen grains, *Pulsatilla*

Introduction

The morphology of pollen grains is a valuable taxonomic feature. Morphological variability of pollen in basal dicotyledons, which include the Ranunculaceae family, has been shown in a number of studies (Praglowski 1975; Nowicke, Skvarla 1982; Blackmore et al. 1995; Dettmann 1998; Milne, Martin 1998; Sauquet, Cantrill 2007; Zhang et al. 2017). The appearance of dicotyledons has become an important moment in the evolution of pollen grain traits, namely aperture morphology, shape, size, and symmetry (Fumess et al. 2007; Wortley et al. 2015).

Ranunculaceae is a large family of angiosperms, the largest in Ranunculales Juss. ex Bercht. et J. Preslis, and includes about 43 genera and more than 2,000 species (Christenhusz, Byng 2016). The family is characterized by 3-colpate, pantocolpate, pantoporate pollen grains, and the sculpture of various shapes, including needle-shaped and reticulate (Erdtman 1953; Kumazawa 1936; Wodehouse 1936; Hamilton 1976; Al-Eisawi 1986; Clarke et al. 1991).

Pulsatilla Mill. (pasque flower) (about 35-45 species) is a genus of herbaceous polycarpic plants native to the temperate latitudes of the northern hemisphere. The taxonomy of the genus is very complex, and several systems have been formed throughout the history of study (Zamels, Paegle 1927; Tamura 1991; Grey-Wilson 2014; Sramko et al. 2019). Intrageneric taxonomy often included data on pollen morphology (Gray-Wilson 2014). Morphology of the pollen grains of *Pulsatilla* and *Anemone* L. (a genus in which some researchers include *Pulsatilla*) is similar, but differs well in the presence of a cushion-shaped protrusion at the spines on the outer wall of the pollen of *Pulsatilla* (Xi, 1985).

Palynological studies of the genus have been performed several times using light microscopy (LM) (Huynh 1970; Nakamura 1980; Wang et al. 1995). According to the apertures, several types of pollen grains are distinguished in *Pulsatilla*. K.-L. Huynh (1970) studied some species of *Pulsatilla*. Among them, *Pulsatilla alpina* (L.) Delarbre, widespread in Central and Southern Europe, and *P. aurea* (Sommier et Levier) Juz. – endemic to the Caucasus, and *P. taraoi* (Makino) Takeda – endemic to the Kuril Islands, had pantocolpate pollen grains. Pantoporate pollen was found in *P. albana* Bercht. et J. Presl, *P. armena* (Boiss.) Rupr. (Southwest Asia) and Asian – *P. campanella* Fisch. ex Regel, *P. tenuiloba* (Hayek) Juz. Most of the species had 3-colpate pollen – *P. kostyczewii* (Korsh.) Juz. (Kyrgyzstan), *P. occidentalis* Freyn (North America), European species – *P. patens* (L.) Mill., *P. vernalis* (L.) Mill., *P. vulgaris* Mill., *P. grandis*, *P. halleri* Willd., *P. rubra* Delarbre, *P. pratensis* (L.) Mill., *P. montana* Rchb., Asian species – *P. turczaninovii* Krylov et Serg., *P. chinensis* (Bunge) Regel, *P. dahurica* (Fisch. ex DC.) Spreng., *P. cernua* (Thunb.) Bercht. et J. Presl. According to Huynh (1970), most species in the genus have 3-colpate pollen, and only a few have pantocolpate or pantoporate pollen grains.

Scanning electron microscopy (SEM) of the genus was performed by Y.-Z. Xi (1985). Data for European species of *Pulsatilla* can be found in the PalDat database (Halbritter 2005) and in the works of Clarke et al. (1991), for Japanese plants – see Miyoshi et al. (2011) and Fujiki et al. (2016).

Y-Z Xi, when processing 12 Asian and 6 European species of the genus, also found 3-colpate, pantocolpate, and pantoporate pollen grains, as well as spiral-apertured and dimorphic (Xi 1985). On the basis of the data obtained, Xi proposed to divide the pollen grains of *Pulsatilla* into 4 types:

1. Dimorphic. It includes 3- and 2-colpate pollen grains. This type is characteristic of *P. kostyczewii*, the analysis of which revealed these two types of pollen in a ratio of 60 % / 40 %, respectively.
2. 3-colpate. The main type, found in most species.
3. Pantocolpate. It occurs in European species and less frequently in Asian species.
4. Pantoporate, including spiral-apertured. Mostly European species, rarely Asian.

It was assumed that the evolutionary trend of pollen types in *Pulsatilla* went from 3-colpate to pantocolpate and then from it to pantoporate (Xi 1985). Apparently, the presence of three or more pollen holes can provide a selective advantage due to the increased fertilization rate (Furness, Rudall, 2004).

Several pollen studies have been devoted to the species *P. cernua* (Iwanami, Yamada 1984; Xi 1985; Miyoshi et al. 2011; Fujiki et al. 2016; Sarwar, Takahashi 2023). This was necessary due to the different results obtained. Iwanami and Yamada described pollen grains of *P. cernua* as a 3-colpate micro-spinate. Sarwar and Takahashi (2023) published a similar result. Y.-Z. Xi considered the pollen shape to be oblate or subsferoidal. According to the results of Miyoshi with coauthors and Fujiki with coauthors, the grains were 3-colpate microreticulate (up to reticulate) of a spheroidal shape. Differences in sizes were also observed: 19 × 16–22 microns (Miyoshi et al. 2011; Fujiki et al. 2016), 31.3 × 33.1 microns (Xi, 1985) and 26 × 23 (Sarwar, Takahashi 2023). We have also included this species in our research.

In total, pollen grains are available for 30 species of *Pulsatilla*, while data are not available for a number of species. In our study, we focus on such species that grow in the Altai Mountain Country and are grouped into the *Pulsatilla* series *Bungeanae*. In addition to them, we studied several other species.

Materials and methods

In our work, we studied the morphology of pollen grains of 19 species of *Pulsatilla*. Samples were taken from the herbarium specimens of the South Siberian Botanical Garden (ALTB), samples of *P. herba-somnii* – from the herbarium of the Siberian Federal University (KRSU), samples of the *P. archarensis* – from the herbarium of the Khingan Nature Reserve (ARKH). The species are divided into sections and se-

ries according to the classification of Grey-Wilson (2014). The location of the samples studied is shown in Table 1.

The surface of pollen grains was observed using scanning electron microscopy (SEM). Pollen was taken from mature anthers, fixed on double-sided adhesive tape, and coated with gold palladium dusting. The spores were photographed and measured using a Hitachi S3400 scanning electron microscope at 10-30 kV at magnifications of 1000, 2000, and 7000 times at the Center for Collective Use of the Institute of Water and Environmental Problems SB RAS (Barnaul, Russia).

The shape and aperture of the pollen grains were determined using Meyer-Melikyan et al. (1999). The surface sculpture is given according to Bucher et al. (2004). The size along the longest axis was divided into groups: S – ≤ 30 microns, M – 31-40 microns, L – > 40 microns.

Table 1. The location of the samples studied

		Species	Location
P. subg. *Kostyczewianae*		*P. kostyczewii*	Kyrgyzstan, Alai district, 3 km up the river Kyzyl-Suu from the mouth of the river. Kashka-Suu, right bank of the river. Kyzyl-Suu.
P. subg. *Pulsatilla* sect. *Pulsatilla*	ser. *Bungenae*	*P. astragalifolia* Pobed.	Mongolia, Kobdos aimak, ridge Altan-Khukhiin-Nuru, 6.5 km southeast of the city of Ulyn-Khuren. 48°30′50″N, 91°59′57″E.
		P. bungeana C. A. Mey. ex Ledeb.	Russia, Republic of Altai, Kosh-Agach district, Yustyd river valley, NE of the former village of Aktal. 49°55′03″N, 88°54′38″E.
		P. neobungeana Zaikov et Shmakov	Russia, Republic of Altai, Kosh-Agach district, SW part of Chuyskaya steppe, the left bank of the Tarkhata river at its exit from the mountains. 49°47′N, 88°32′E.
		P. neobungeana	Russia, Republic of Altai, Kosh-Agach district, Kurai ridge, southern macroslope of Tobozhok mountain, in the upper reaches of the Koksair tract. 50°03′38″N, 88°48′34″E.
		P. herbertii Zaikov et Shmakov	Mongolia, Khovd aimag, Zhargalant-Khairkhan (Zuun-Zhargalant) ridge, Ar-Shaatyn-Gol river valley. 47°44′N, 92°27′E.
		P. tenuiloba	Mongolia, Khubsugol aimak, left bank of the river Selenga, Dolodyn-Nuru ridge, southern part. 49°18′16″N, 100°54′57″E.
	ser. *Pulsatilla*	*P. herba-somnii* Stepanov	Russia, Krasnoyarsk environs, "Beautiful coast".
		P. turczaninovii x *P. multifida*	Russia, Republic of Altai, Chemal district, Katun river valley, 2 km south of Chemal village. 51°10′N, 86°07′E.

		Species	Location
		P. turczaninovii	Russia, Republic of Altai, Chemal district, Katun river valley, 2 km south of Chemal village. 51°10′N, 86°07′E.
		P. archarensis	Amur region, Arkharinsky district, 11 km south of the village of Arkhara.
		P. taurica Juz.	Russia, Crimea, Karadag Nature Reserve, Mount Legener.
	ser. *Albanae*	*P. ambigua* (Turcz.) Juz.	Russia, Republic of Buryatia, Tukinsky district, Irkut River valley, 2.5 km southeast of the village Moygots. 51°38′32″N, 101°25′55″E.
		P. campanella	Russia, Republic of Altai, Kosh-Agach district, Kurai ridge, southern macroslope of Tobozhok mountain, in the upper reaches of the Koksair tract. 50°03′46″N, 88°49′35″E.
		P. campanella	Kyrgyzstan, Alai district, Kek-Suu river basin, 1 km east of Ikizyak village.
		P. andina (Rupr.) Grossh.	Russia, Dagestan, Khunzakh district, near the village Mochoh.
P. subg. *Pulsatilla* sect. *Patentes*		*P. multifida* (E. Pritz.) Juz.	Russia, Republic of Altai, Chemal district, near the village of Tolgoek. 51°19'N, 86°01'E.
		P. kryloviana Juz.	Russia, Altai Territory, Uglovsky district, 4 km northwest of the village of Krugloe. 51°18′17″N, 80°22′59″E.
P. subg. *Pulsatilla* *P.* sect. *Semicampanaria*		*P. cernua*	Russia, Primorsky Krai, Khasansky district, 3 km south of the village Andreevka, Troitsa Bay. 42°08′N, 131°09′E.
		P. dahurica	Russia, Primorsky Krai, Suchansky district, Narechnaya station district.

Results and discussion

We have studied the morphology from pollen grains of 19 species of Pulsatilla. The sculpture of the pollen grains of all the species was micro spinate. The surface was smooth and rarely tuberculate. The shape of the pollen grains varied from elongated-ellipsoidal to spheroidal. The dimensions along the longest axis ranged from 17 microns to 45 microns, depending on the species. Summarized data are in Suppl. material 1: Table 2S and Suppl. material 2: Table 3S.

Most of the species had a 3-colpate aperture (Fig. 1).

All species had an elongated-ellipsoidal or ellipsoidal shape with a smooth surface, except for *P. cernua* and *P. dahurica* (spheroidal or oblate spheroidal; the surface is tuberculate). The pollen grains of two closely related species, *P. astragalifolia* and *P. bungeana*, differed only in the size of the longest axis.

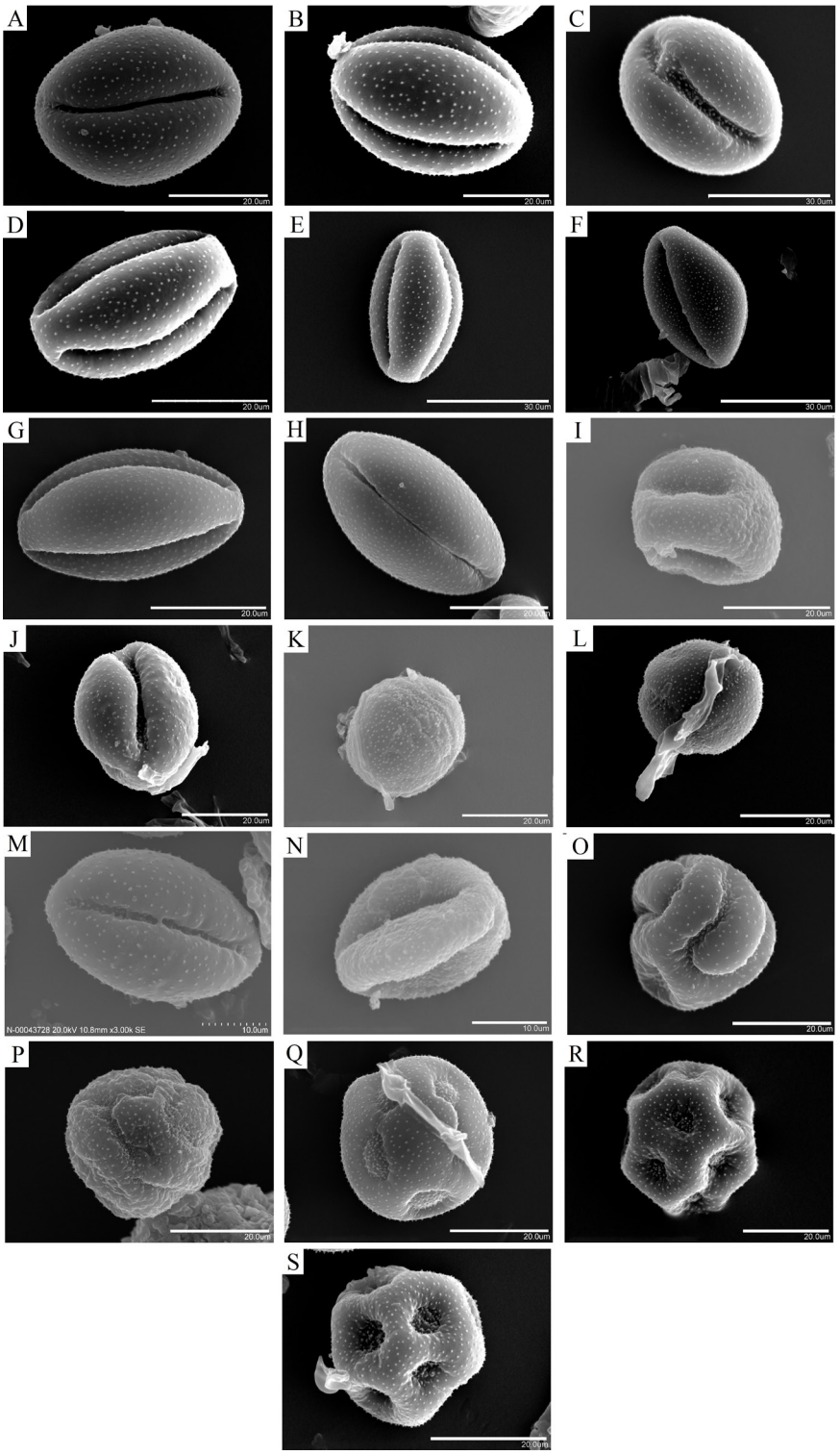


Figure 1. Description is on the next page.

Figure 1. SEM micrographs of pollen grains of species (microns): **A** – *P. multifida*, **B** – *P. kryloviana*, **C** – *P. herba-somnii*, **D** – *P. turczaninovii* × *P. multifida*, **E** – *P. turczaninovii*, **F** – *P. ambigua*, **G** – *P. astragalifolia*, **H** – *P. bungeana*, **I** – *P. ajanensis*, **J** – *P. taurica*, **K** – *P. cernua*, **L** – *P. dahurica*, **M** – *P. archarensis*, **N** – *P. kostyczewii*, **O** – *P. herbertii*, **P** – *P. tenuiloba*, **Q** – *P. neobungeana*, **R** – *P. campanella*, **S** – *P. andina*, *P. astragalifolia*, *P. bungeana*, *P. ajanensis*, *P. cernua*, *P. ambigua*, *P. taurica*, *P. turczaninovii* × *P. multifida*, *P. kryloviana*, *P. herba-somnii*, *P. turczaninovii*, *P. arcaharensis*, *P. multifida*.

The study of the pollen of *P. cernua* confirmed the data of Y. Iwanami, Y. Yamada and A. K. M. G. Sarwar, H. Takahashi, according to which the pollen grains are microspinate. The sizes of pollen grains of some species we studied differed from previous research. Different data were also obtained for *P. dahurica*, *P. ambigua*, and *P. cernua*. The pollen sizes of *P. campanella* collected in the Republic of Altai were 32.25 microns in size; Xi has revealed the close result for this species – 33.1 microns. Meanwhile, the samples collected in Kyrgyzstan were smaller, 23.8 microns. This may be due to various processes during drying of the material. For greater precision, the sampling for each species should be increased.

The genetically close species *P. kryloviana* and *P. multifida* differed in both the shape of the pollen grains and their sizes.

The type of spiral aperture pollen grains was shown in two species: *P. tenuiloba* and *P. herbertii*. The species *P. astragalifolia*, *P. bungeana*, *P. neobungeana*, and *P. herbertii* until recently were taken as one and determined by us based on morphological and molecular genetic data (Zaikov et al. 2024) also differ in the morphology of pollen grains. Pantoporate pollen grains were in *P. neobungeana*, 3-colpate in *P. astragalifolia* and *P. bungeana*, pantocolpate in *P. herbertii* and *P. tenuiloba*. The relationship of the latter two species was also genetically confirmed.

The type of pantoporate pollen grains combined the species *P. campanella*, *P. andina*, and *P. neobungeana*. The latter is probably closer to *P. campanella* and should be transferred to the series Albanae, but this requires additional morphological and molecular genetic evidence. *P. ambigua*, referred to in this series, is close to *P. turczaninovii* in morphological features and pollen morphology. Based on the data obtained, assignment of species to one section or another should be reviewed.

Descriptions and photographs of pollen grains are presented below.

1. *Pulsatilla ajanensis* Regel et Tiling
   Fig. 2

Pollen grains have an oblate spheroidal shape. Sizes (23–27.2)25.08 × 28.46(27.1–29) microns. The size along the longest axis is S. The aperture is 3-colpate, and the furrows are clearly expressed, with spines. The sculpture is micro spinate, the width of the base of the spines is 0.2–0.4 microns, the height is about 0.2–0.4 microns, sparsely and evenly distributed. The surface is tuberculate and strongly perforated.

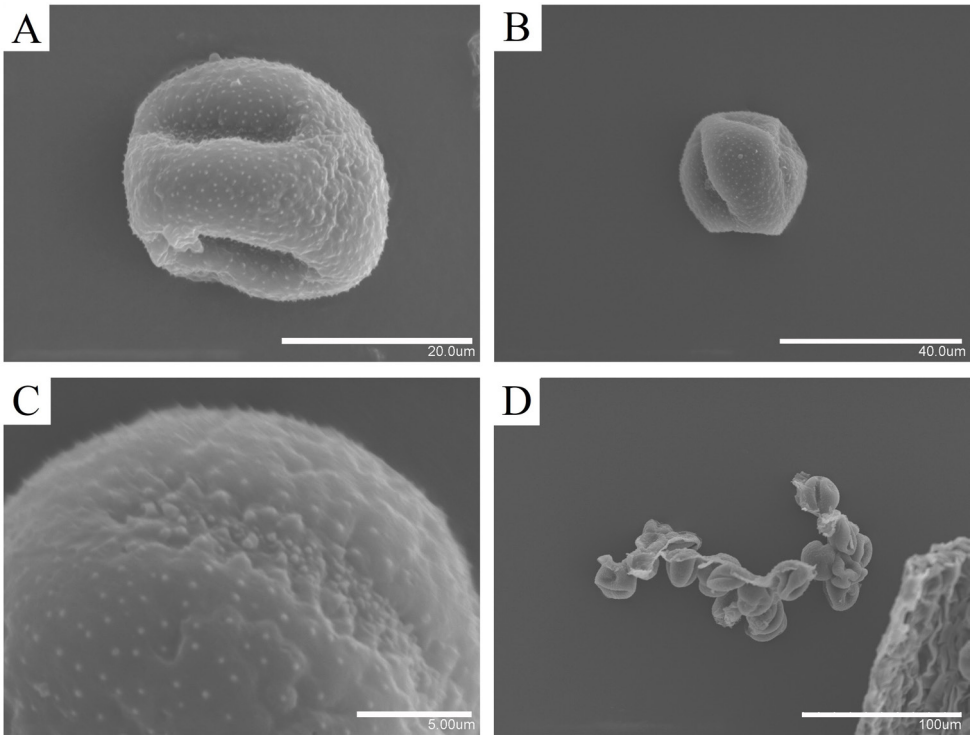


Figure 2. SEM micrographs of the pollen grains of *P. ajanensis*: appearance (**A**, **B**, **D**), surface (**C**) (micron).

2. *Pulsatilla ambigua* Turcz. ex Pritz.
   Fig. 3

Pollen grains have an elongated shape. Sizes (31.2–37.2)34.46 × 22.23(20.5–24.2) / (27.8–33)29.5 × 31.3(29.5–34.8) microns (Xi, 1985). The size along the longest axis is M. The aperture is 3-colpate, and the furrows are clearly expressed, with spines. The sculpture is micro-spinate, the width of the base of the spines is 0.2–0.3 microns, the height is approximately 0.2-0.3 microns, evenly distributed. The surface is smooth.

3. *Pulsatilla andina* (Rupr.) Grossh.
   Fig. 4

Pollen grains have a spheroidal shape, (26.3–31.1)29.02 microns in diameter. The size along the longest axis is S. The aperture is pantoporate. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.3–0.4 microns, evenly distributed. The surface is smooth.

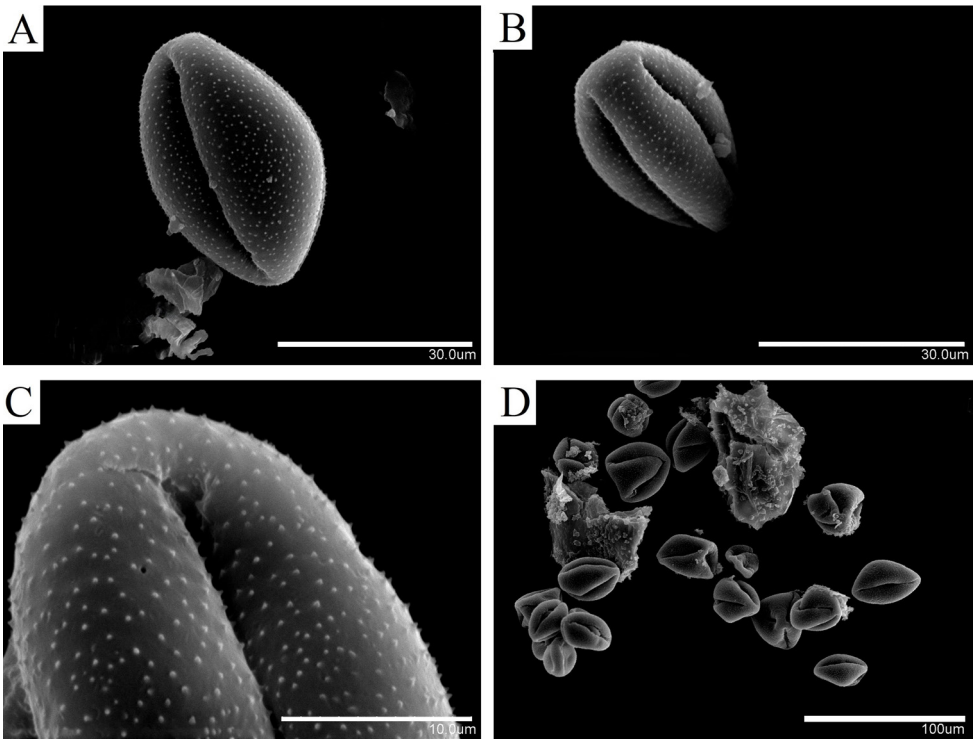


Figure 3. SEM micrographs of the pollen grains of *P. ambigua*: appearance (**A**, **B**, **D**), surface (**C**) (micron).

4. *Pulsatilla archarensis* Kudrin
Fig. 5

Pollen grains have an elongated ellipsoidal shape. Sizes (17.8–23.3)22.2 × 31.5(29.3–33.5) microns. The size along the longest axis is M. The aperture is 3-colpate, the furrows are clearly expressed and are long. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.3–0.4 microns, evenly distributed. The surface is smooth.

5. *Pulsatilla astragalifolia* Pobed.
Fig. 6

Pollen grains have an elongated ellipsoidal shape. Sizes (20.7–23.5)21.78 × 36.31(35–39.6) microns. The size along the longest axis is M. The aperture is 3-colpate, the furrows are clearly expressed and are long. The sculpture is micro spinate, the width of the base of the spines is 0.2 to 0.4 microns, the height of the spines is approximately 0.2 microns. They are densely and evenly distributed. The surface is smooth.

6. *Pulsatilla bungeana* C.A. Mey. ex Ledeb.
Fig. 7

Pollen grains have an elongated ellipsoidal shape. Sizes (25–26.3)25.42 × 41.46 (41–43.3) microns. The size along the longest axis is L. The aperture is 3-colpate, the furrows are clearly expressed and are long. The sculpture is micro-spinate, the width of the base of the spines is 0.2 microns, the height is approximately 0.3–0.4 microns. They are densely and evenly distributed. The surface is smooth.

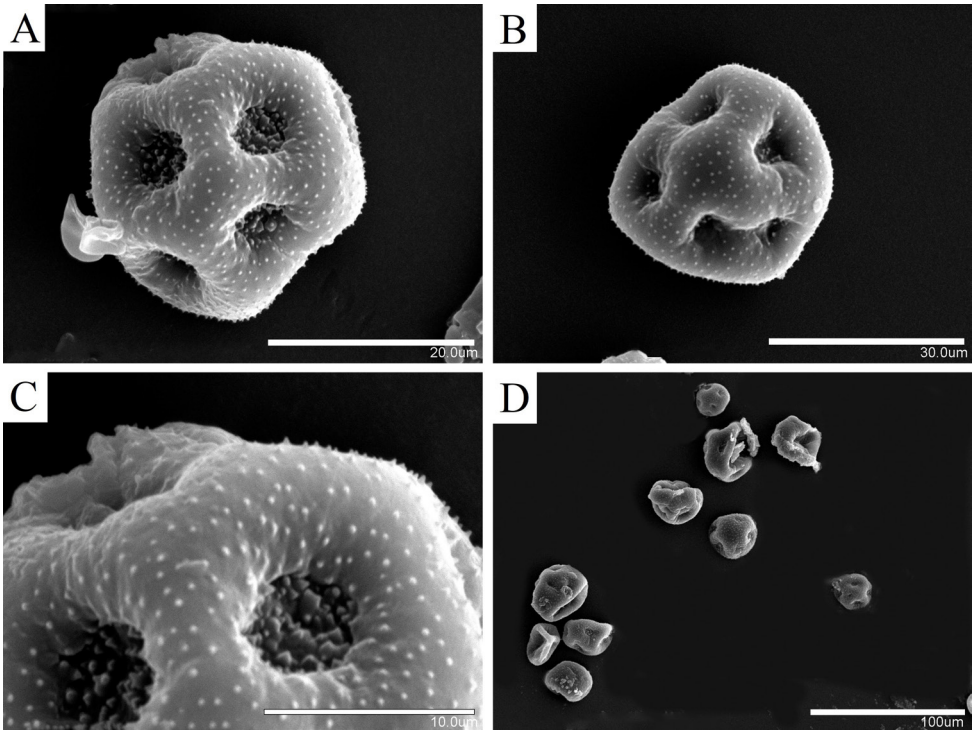


Figure 4. SEM micrographs of the pollen grains of *P. andina*: appearance (**A**, **B**, **D**), surface (**C**), (micron).

7. *Pulsatilla campanella* Fisch. ex Regel
Fig. 8

Pollen grains have a spheroidal shape. The diameter varied for collections from different locations (30.3–35.4)32.25 microns (Republic of Altai) / (20.7–24.9)23.8 microns (Kyrgyzstan) / 33.1(29.6–34.8) microns (Xi, 1985). The size along the longest axis is S, M. The aperture is pantoporate. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.3–0.4 microns, evenly distributed. The surface is smooth and highly perforated.

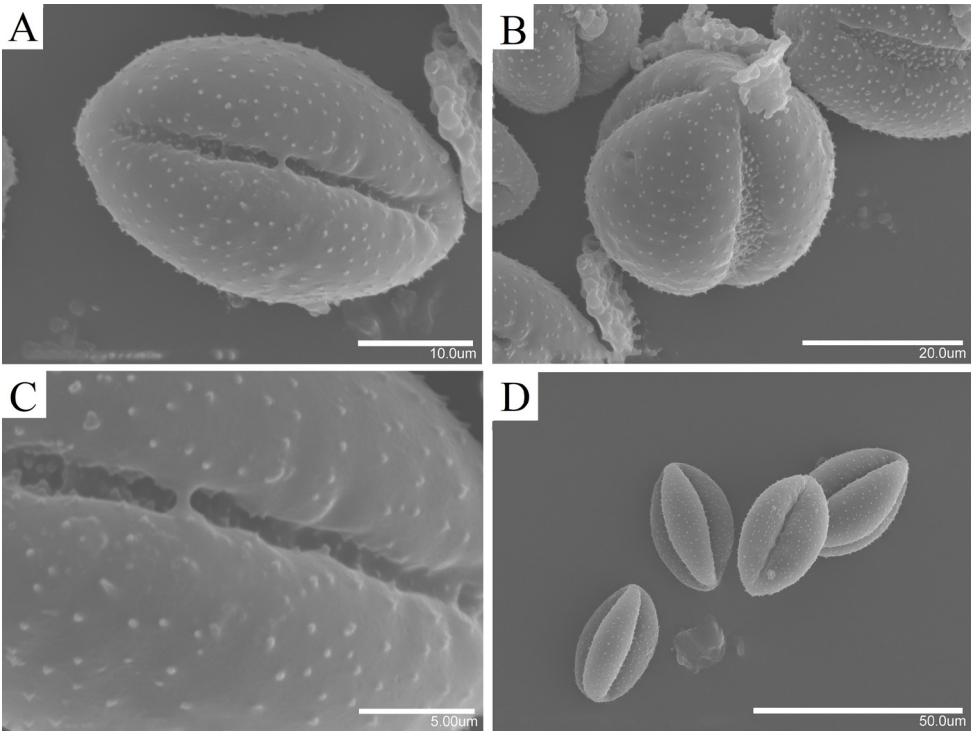


Figure 5. SEM micrographs of the pollen grains of *P. archarensis*: appearance (**A**, **B**, **D**), surface (**C**) (micron).

8. *Pulsatilla cernua* (Thunb.) Bercht. et J. Presl
Fig. 9

Pollen grains have a spheroidal shape, rarely elongated. Dimensions (23–28.4)25.38 microns (our data) / (21.9–25.6)22.8 × 33.05(30.5–33.9) microns / 6 × 19 × 16–22 microns (Miyoshi et al., 2011; Fujiki et al., 2016) / 31.3 × 33.1 microns (Xi, 1985) / 26 × 23 (Sarwar, Takahashi, 2023). The size along the longest axis is S, M. The aperture is 3-colpate; the furrows are weakly expressed, with spines. The sculpture is micro-spinate, the width of the base of the spines is 0.2–0.3 microns, the height is approximately 0.2-0.3 microns, evenly distributed. The surface is tuberculate.

9. *Pulsatilla dahurica* (Fisch. ex DC.) Spreng.
Fig. 10

Pollen grains have a spheroidal shape and are rarely slightly elongated. Sizes (23–28.1)25.4 microns (our data) / 31.3(27.8–33.1) microns (Xi, 1985). The size along the longest axis is S. The aperture is 3-colpate or pantocolpate; the furrows are not always clearly expressed, with spines. The sculpture is micro-spinate, the width of

the base of the spines is 0.3 microns, the height is about 0.3 microns, evenly distributed. The surface is tuberculate, with a well-defined perforation.

10. Pulsatilla herba-somnii Stepanov
Fig. 11

The pollen grains have an ellipsoidal shape. Sizes (29.2–34.6)33.39 × 41.56(41–44.8) microns. The size along the longest axis is L. The aperture is 3-colpate, the furrows are clearly expressed and are long. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.5 microns, the height is approximately 0.3-0.5 microns, evenly distributed. The surface is smooth.

11. Pulsatilla herbertii Zaikov et Shmakov
Fig. 12

Pollen grains have an oblate spheroidal shape. Sizes (30–34.6)34 × 27.7(24.1–29.9) microns. The size along the longest axis is M. The aperture is spiral, and the furrows are clearly expressed, long, with spines. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.3–0.4 microns. They are sparsely and evenly distributed. The surface is smooth.

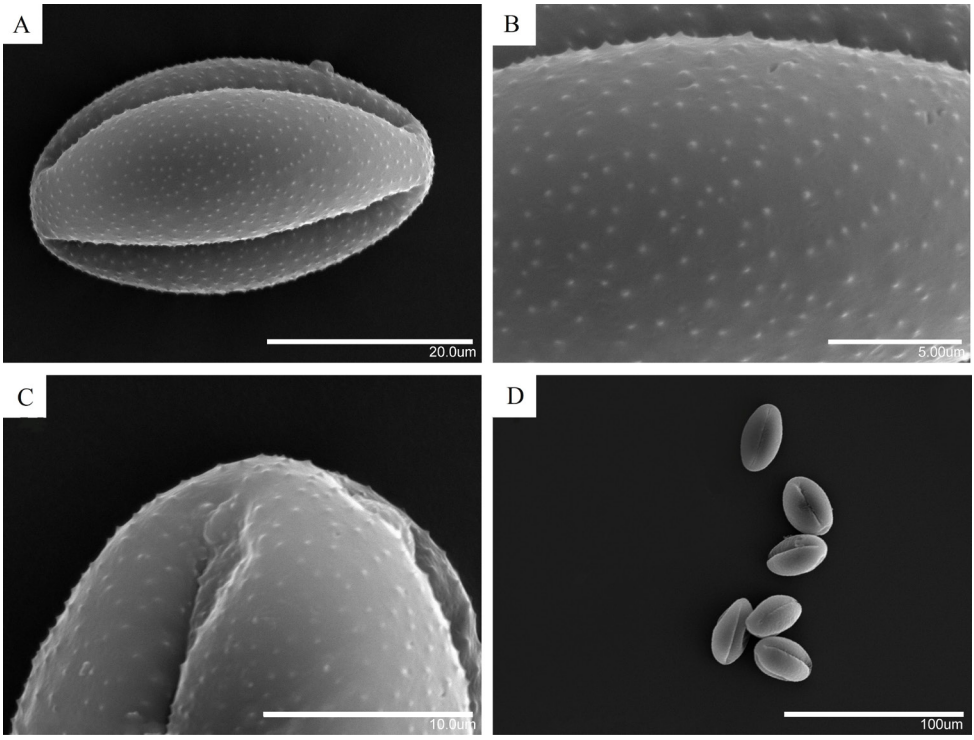


Figure 6. SEM micrographs of the pollen grains of *P. astragalifolia*: appearance (**A**, **D**); surface (**B**, **C**) (micron).

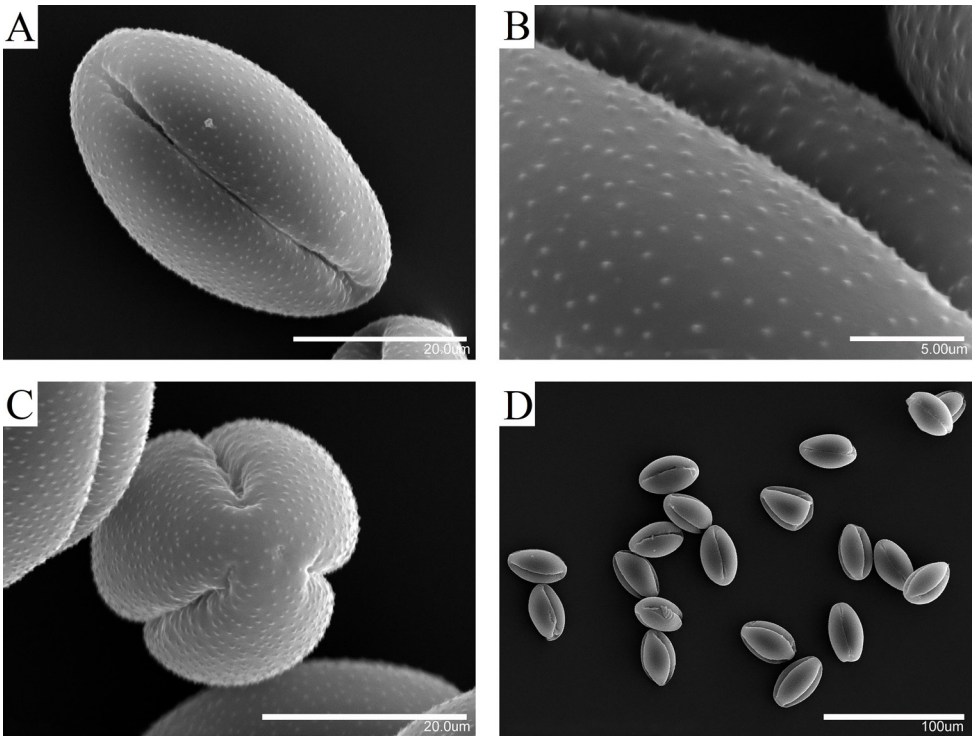


Figure 7. SEM micrographs of the pollen grains of *P. bungeana*: appearance (**A**, **C**, **D**), surface (**B**) (micron).

12. Pulsatilla kostyczewii (Korsh.) Juz.
Fig. 13

Pollen grains have an oblate spheroidal shape. Sizes (17.4–19.8)18.37 × 23.8(22.3–25.6) microns / (17.4–27.8)26.1 × 24.3(17.4–27.8) (Xi, 1985). The size along the longest axis is S. The aperture is 3-colpate, rarely 2-colpate; the furrows are implicitly expressed, with large spines. The sculpture is micro-spinate, the width of the base of the spines is 0.3 microns, the height is approximately 0.3–0.4 microns, sparsely distributed. The surface is tuberculate.

13. Pulsatilla kryloviana Juz.
Fig. 14

The pollen grains have an ellipsoidal shape. Sizes (32–35.6)33.77 × 45.76(43.9–47.8) microns. The size along the longest axis is L. The aperture is 3-colpate, the furrows are clearly expressed and are long. The sculpture is micro spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.4–0.5 microns, evenly distributed. The surface is smooth with perforation.

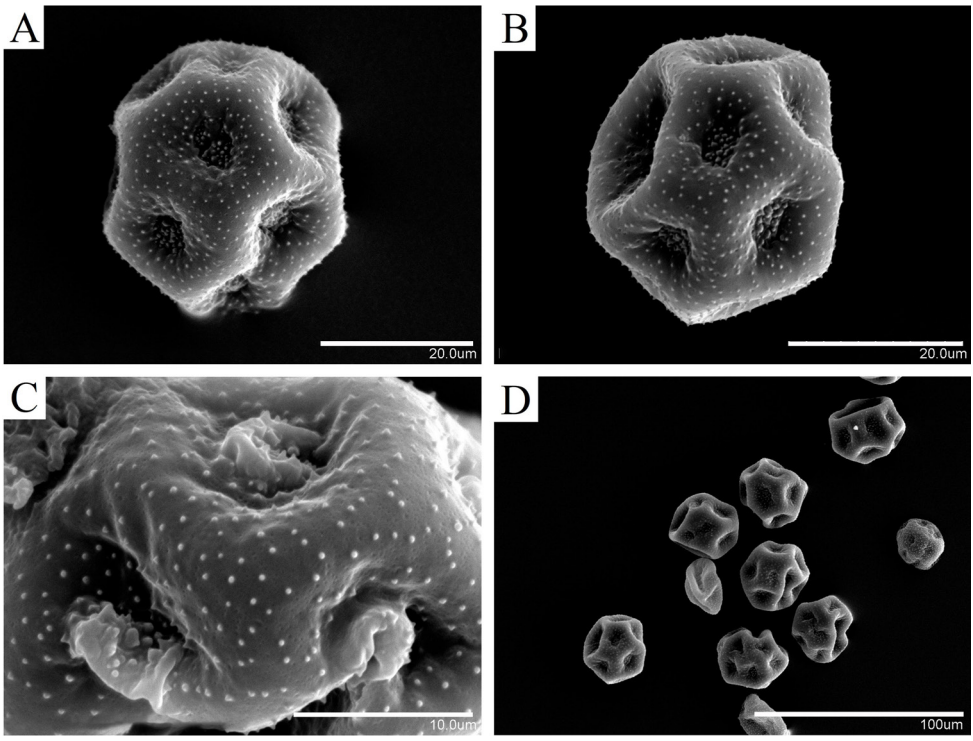


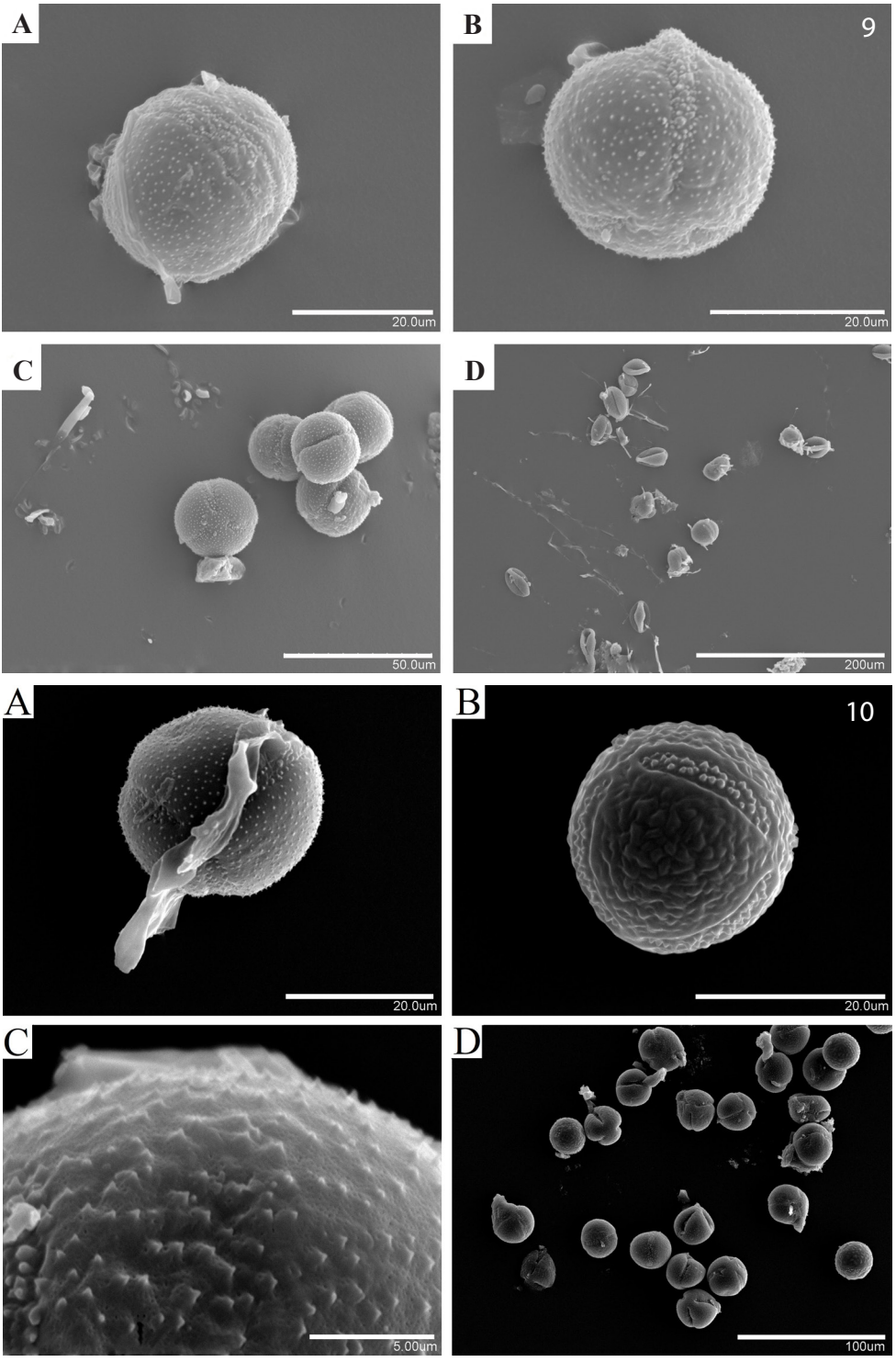
Figure 8. SEM micrographs of the pollen grains of *P. campanella*: appearance (**A**, **B**, **D**), surface (**C**) (micron).

14. Pulsatilla multifida (E. Pritz.) Juz.
Fig. 15

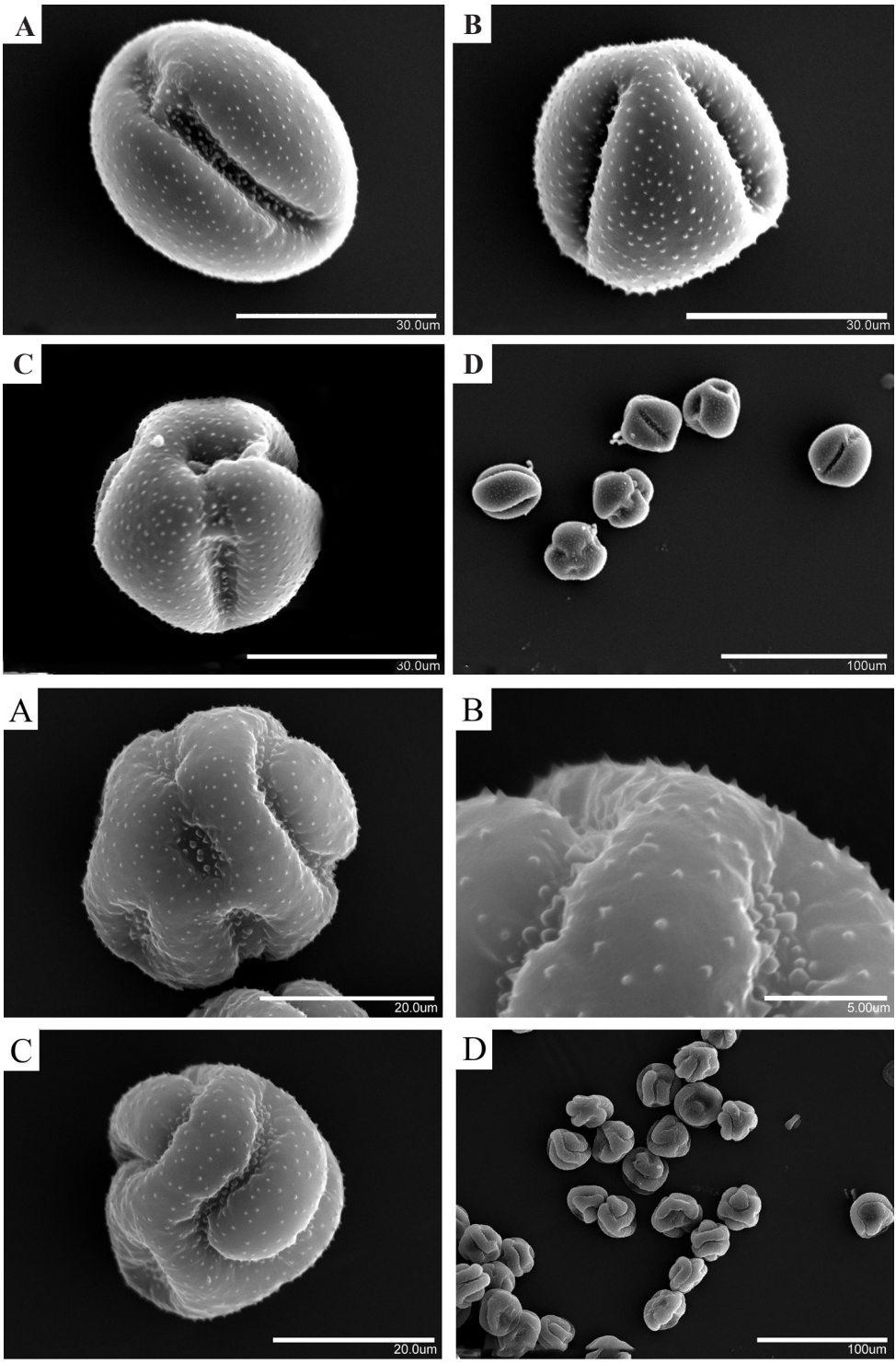
The pollen grains have an ellipsoidal shape. Sizes (31.3–35.9)33.84 × 41.7(39.6–43.6) microns (our data)/ 38.3(33.1–41.7) × 48.7(46.9–53.9) microns (Xi, 1985). The size along the longest axis is M. The aperture is 3-colpate, the furrows are clearly expressed and are long. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.3–0.4 microns, evenly distributed. The surface is smooth.

15. Pulsatilla neobungeana Zaikov et Shmakov
Fig. 16

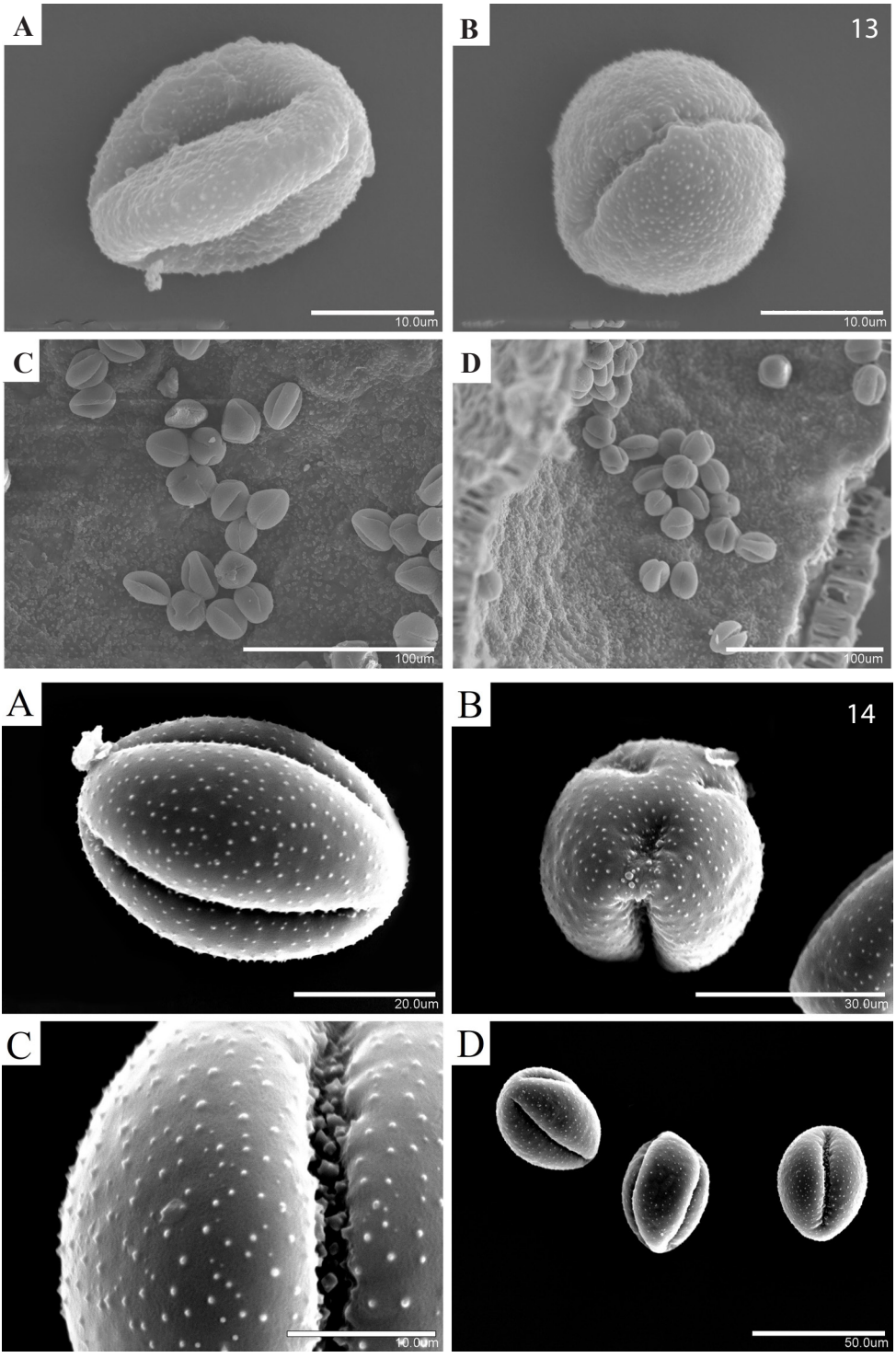
Pollen grains have a spheroidal shape, rarely oblate, (27.2-37.5), 31.04 microns in diam. The size along the longest axis is M. The aperture is pantoporate, and samples with pantocolpate are rarely found. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.3–0.4 microns. They are sparsely and evenly distributed. The surface is smooth.



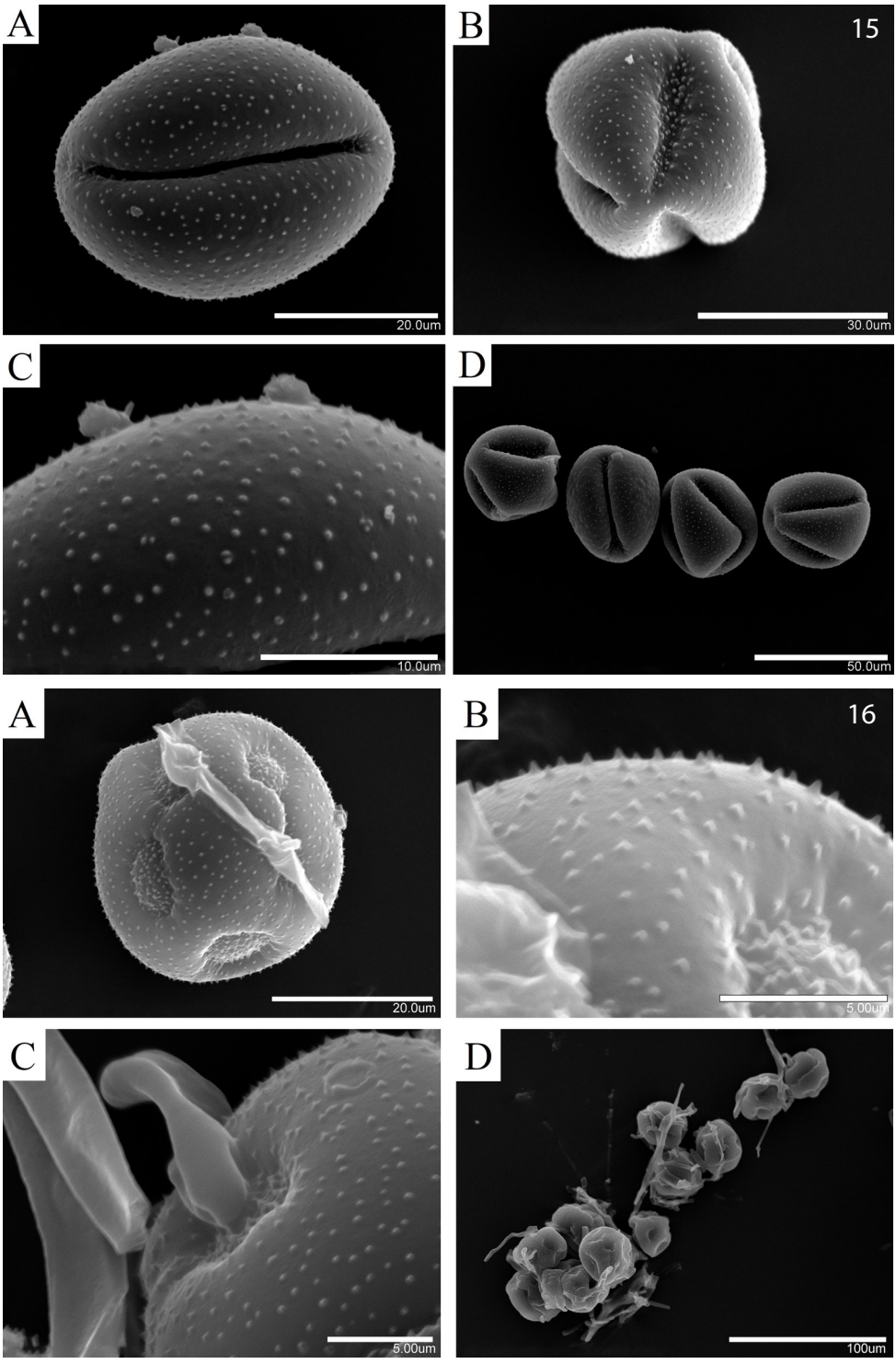
Figures 9–10. SEM micrographs of the pollen grains. **9** – *P. cernua* (micron); **10** – *P. dahurica*: appearance (**A**, **B**, **D**), surface (**C**) (micron).



Figures 11–12. SEM micrographs of the pollen grains. **11** – *P. herba-somnii*; **12** – *P. herbertii*: appearance (**A**, **C**, **D**), surface (**B**) (micron).



Figures 13–14. SEM micrographs of the pollen grains. **13** – *P. kostyczewii* (micron); **14** – *P. kryloviana*: appearance (**A**, **B**, **D**), surface (**C**) (micron).



Figures 15–16. SEM micrographs of the pollen grains. **15** – *P. multifida*: appearance (**A**, **B**, **D**), surface (**C**) (micron); **16** – *P. neobungeana*: appearance (**A**, **C**, **D**), surface (**B**) (micron).

16. *Pulsatilla taurica* Juz.
Fig. 17

The pollen grains have an ellipsoidal shape. Sizes (25.2–30.3)28.33 × 36.12(32.6–38.3) microns. The size along the longest axis is M. The aperture is 3-colpate, the furrows are clearly expressed and are long. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.3–0.4 microns, evenly distributed. The surface is smooth with well-defined perforation.

17. *Pulsatilla tenuiloba* (Hayek) Juz.
Fig. 18

Pollen grains have a spheroidal or oblate spheroidal shape, (25.9–30.5)28.31 microns in diameter. The size along the longest axis is M. The aperture is spiral, the furrows are not always clearly expressed, long, with spines. The sculpture is micro-spinate, the width of the base of the spines is 0.3 microns, the height is approximately 0.3–0.4 microns. They are sparsely and evenly distributed. The surface is tuberculate.

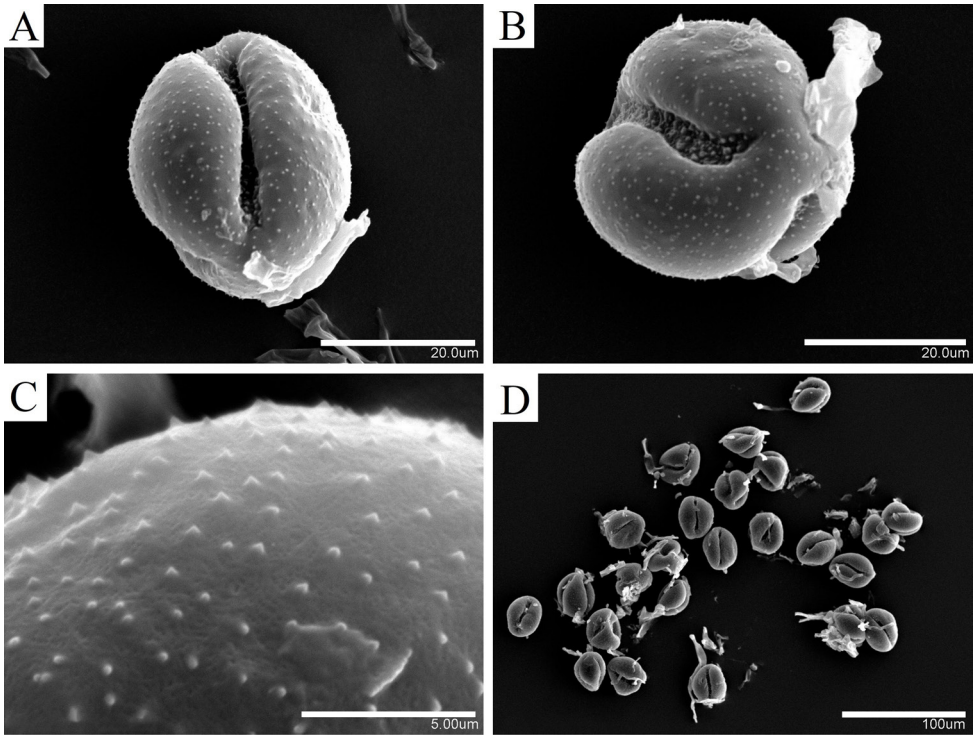


Figure 17. SEM micrographs of the pollen grains of *P. taurica*: appearance (**A**, **B**, **D**), surface (**C**) (micron).

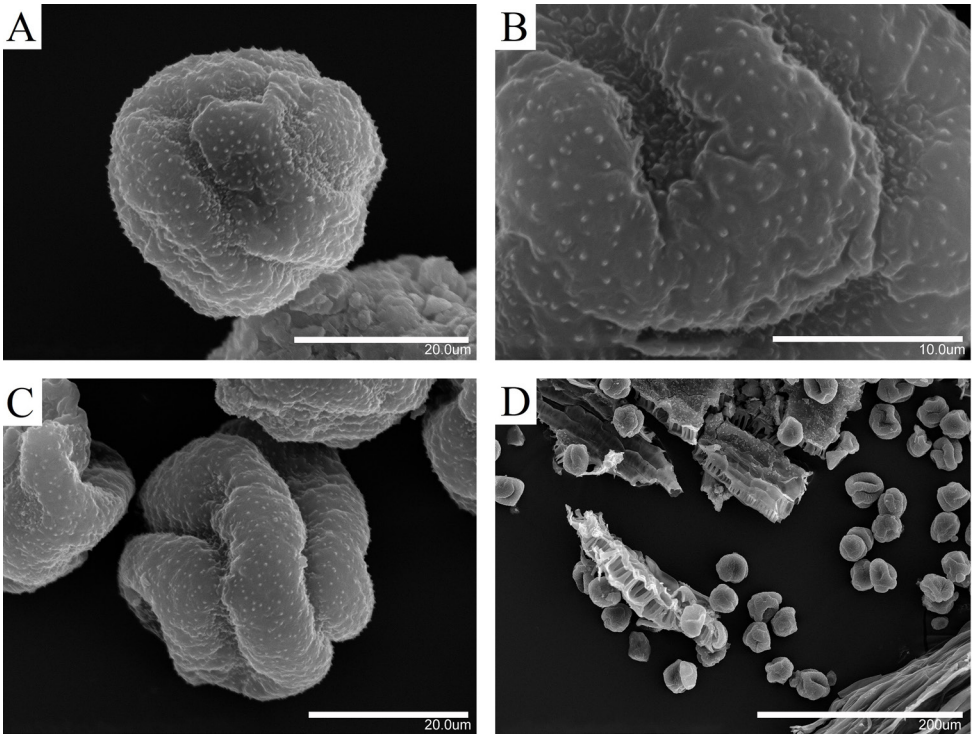


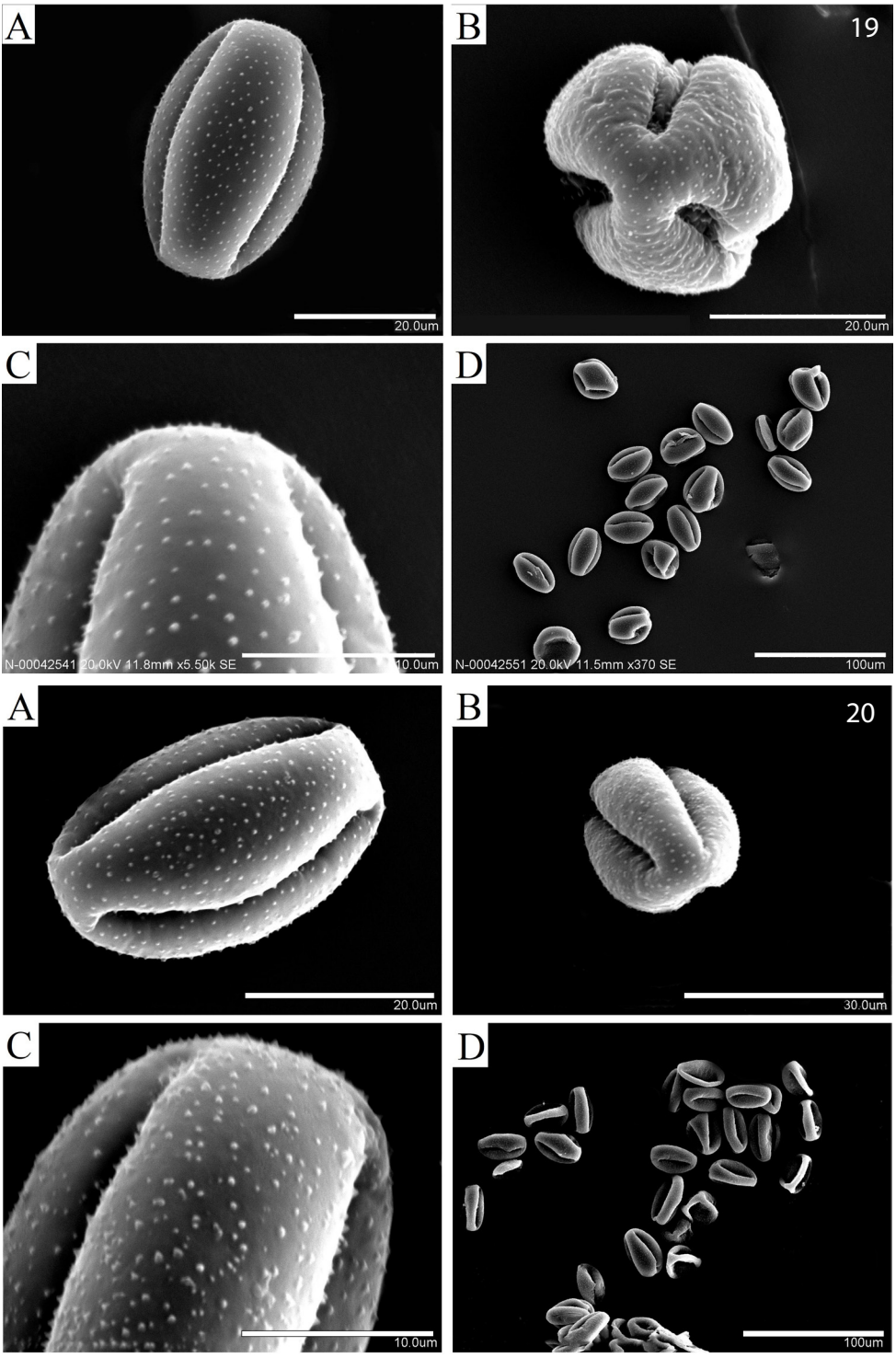
Figure 18. SEM micrographs of the pollen grains of *P. tenuiloba*: appearance (**A**, **C**, **D**), surface (**B**) (micron).

18. *Pulsatilla turczaninovii* Krylov et Serg.
Fig. 19

Pollen grains have an elongated ellipsoidal shape. Sizes (22.3–27.2)21.18 × 34.12(35.3–38.9) microns / (28.3–38.3)34.8 × 34.8(27.8–38.3) microns (Xi, 1985). The size along the longest axis is M. The aperture is 3-colpate, the furrows are clearly expressed and are long. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.3–0.4 microns, evenly distributed. The surface is smooth.

19. *Pulsatilla turczaninovii* Krylov et Serg. × *P. multifida* (E. Pritz.) Juz.
Fig. 20

Pollen grains have an elongated ellipsoidal shape. Sizes (17.1–21.9)18.93 × 32.21(30.3–34.7) microns. The size along the longest axis is M. The aperture is 3-colpate, the furrows are clearly expressed and are long. The sculpture is micro-spinate, the width of the base of the spines is 0.3–0.4 microns, the height is about 0.3–0.4 microns, and they are unevenly distributed. The surface is smooth.



Figures 19–20. SEM micrographs of the pollen grains. **19** – *P. turczaninovii*: appearance (**A**, **B**, **D**), surface (**C**) (micron); **20** – *P. turczaninovii* × *P. multifida*: appearance (**A**, **B**, **D**), surface (**C**) (micron).

Conclusion

This study represents the first comprehensive investigation of the pollen grain morphology of nine *Pulsatilla* species. By synthesizing data from previous research alongside the present findings, pollen grains of *Pulsatilla* can be classified into the following distinct types:

1. Dimorphic (*P. kostyczewii*).
2. Three-colpate (*P. astragalifolia*, *P. bungeana*, *P. ajanensis*, *P. cernua*, *P. ambigua*, *P. taurica*, *P. kryloviana*, *P. herba-somnii*, *P. turczaninovii*, *P. archarensis*, *P. multifida*, *P. scherfelii*, *P. integrifolia*, *P. patens*, *P. vernalis*, *P. vulgaris*, *P. grandis*, *P. halleri*, *P. rubra*, *P. pratensis*, *P. montana*, *P. chinensis*, *P. dahurica*, *P. angustifolia*, *P. orientali-sibirica*).
3. Pantocolpate (including mixed with three-beard) (*P. alpina*, *P. alba*, *P. aurea*, *P. nipponica*, *P. taraoi*, *P. occidentalis*).
4. Spiral apertured (*P. herbertii*, *P. tenuiloba*).
5. Pantoporate (*P. neobungeana*, *P. campanella*, *P. andina*, *P. albana*, *P. sukaczewii*, *P. wallichiana*).

Acknowledgements

The studies were carried out within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation: V. F. Zaykov, A. P. Shalimov, T. A. Sinitsyna, A. A. Kechaikin, S. Yu. Pankratov, A. A. Batkin, and A. I. Shmakov (Altai State University) – project No. FZMW-2023-0008.

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Supplementary material 1

Table 2S. Characteristics of pollen grains of the studied species of *Pulsatilla*

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Data type: tables

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Supplementary material 2

Table 3S. The pollen characteristics of *Pulsatilla* species are classified according to the Grey-Wilson (2014) system, with modifications

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