Article

Pollen Morphology of Some Species from Genus Nitraria

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Abstract: An analysis of pollen grains (in Nitraria sibirica Pall., N. schoberi L., N. komarovii Ilijn & Lava ex Bobrov, and N. pamirica L. Vassil.) was performed on natural material collected in Russia, Kazakhstan, and Tajikistan. Herbarium specimens from the collection at Komarov Botanical Institute, Russian Academy of Sciences (N. tangutorum Bobrov and N. praevisa Bobrov) were examined, too. Pollen grains of two species—N. pamirica and N. praevisa—were studied for the first time. N. tangutorum and N. praevisa were found to have the perprolate pollen shape, whereas N. pamirica was found to have the subprolate shape. An intraspecific differentiation of N. sibirica was noted. Populations of N. sibirica (Taskarasu, Karatal, and Basshi) possess pollen grains of the subprolate or prolate shape, striate and perforate exine ornamentation, and a longer equatorial axis and a shorter polar axis than other specimens of N. sibirica. N. schoberi in all populations had anomalous shapes of some pollen grains. Overall, we demonstrated that the length ratio of the polar axis to the equatorial axis, characteristics of pollen in polar view, colpus morphology, and surface ornamentation of pollen grains in the genus Nitraria are of great taxonomic importance for the identification of species.

Keywords: Nitraria sibirica; Nitraria schoberi; Nitraria komarovii; Nitraria pamirica; Nitraria tangutorum; Nitraria praevisa; pollen structure; light microscopy; scanning electron microscopy

1. Introduction

The genus Nitraria L. (Nitrariaceae) includes more than 10 species and occurs in the steppe, semidesert, and desert regions of Asia, North Africa, Southeastern Europe (Romania), and Australia [1–8].

Despite the small taxonomic size of the genus and more than a century of research history [9–15], researchers do not have a clear idea about the number of Nitraria species, their genesis, and the systematics of the genus. The main reason is the weak isolation of species according to macro- and micromorphological characters, since most of species differ only in the metric characters of leaves and fruits [1,2,16–18].

Modern methods for the analysis of Nitraria species also cannot elucidate their genesis. In particular, with the help of assays of inter simple sequence repeats (ISSR) markers and sequencing of the internal transcribed spacer (ITS) region and of chloroplast DNA spacers in Inner Mongolia, researchers have identified transitional forms between N. tangutorum and N. roborowskii as spontaneous hybrids [19]. The existing phylogenetic and biogeographic analyses of the genus by means of DNA sequencing of six regions in the chloroplast genome and of the ITS region [20] are based mainly on specimens from the collection at the Turpan Botanical Garden (Xinjiang Province, China), not from natural habitats. Type specimens have not been studied by those authors, and information about nucleotide variation of the DNA fragments has not been provided.

Therefore, the use of additional sources of information, such as micromorphological characters, may help taxonomists to better delineate species boundaries within the genus. Palynomorphological data are thought to be some of the important parameters for the identification of various problematic taxa. Polarity, shape, size, aperture structure, symmetry,
and ornamentation of pollen grains are highly conserved traits and are often utilized to solve complex problems of plant taxonomy [21–26].

So, to clarify the independence of the Nitrariaceae family, Agababyan and Tumanyan [27] have used structural features of pollen grains. They provided palynological data on *N. tangutorum, N. sibirica, N. schoberi, N. komarovii, N. sphaerocarpa* Maxim., and *N. retusa* (Forsk.) Aschers., and according to this evidence, these species differ in the size of pollen grains, in the structure of furrows and pores, in the thickness of individual layers of the sporoderm, and in the nature of sculptural elements of the exine. Woutersen et al. [28] have employed morphological parameters of pollen grains in research into *Nitraria* phylogeny. They investigated *N. tangutorum, N. sibirica, N. schoberi, N. komarovii, N. sphaerocarpa, N. retusa,* and *N. billardierei* DC.

Here, we examined the morphology of pollen grains of six species of *Nitraria*, and for two species, such information is reported for the first time. It is noteworthy that the study was conducted on original mass material from natural populations with an assessment of trait variation. This research was designed and conducted with the aim (1) to evaluate the taxonomic significance of pollen features of *Nitraria* taxa and (2) to enlighten the morphology of pollen grains, pollen types using light (LM) and scanning electron microscopy (SEM). We note that in the light of resolution issues of current molecular data, the study of *Nitraria* pollen can assist in the identification of problematic species, which should be covered in any future molecular assessment.

2. Results

Detailed characteristics of pollen grains for the six species of *Nitraria* are provided in Table 1 and Figures 1 and 2. The description of pollen traits for each *Nitraria* species is given below.
Table 1. Measurements (in µm) of pollen grains of *Nitraria*. The data are presented as the mean (X), standard error (Sx), and the coefficient of variation (CV, %).

| No. | Taxa               | Specimen | Polar Axis (P)  | Equatorial Axis (E) | P/E       | Shape                      | Ornamentation |
|-----|--------------------|----------|----------------|---------------------|-----------|----------------------------|---------------|
|     | Range X ± Sx       |            | CV, %          | Range X ± Sx        | CV, %     | Range X ± Sx               | CV, %         | Shape                 | Ornamentation |
| 1   | N. sibirica        | Balhash   | 36.9–44.72  | 42.08 ± 0.44        | 5.2       | 20.30–24.58               | 22.46 ± 0.22  | 4.9                   | 1.65–2.13  | 1.88 ± 0.02          | 6.5 prolate striate |
| 2   | N. sibirica        | Kosh-Agach| 33.82–42.83  | 39.63 ± 0.49        | 6.2       | 20.08–24.71               | 22.26 ± 0.22  | 5.0                   | 1.41–1.99  | 1.78 ± 0.02          | 7.3 prolate striate |
| 3   | N. sibirica        | Dauria    | 27.60–39.37  | 34.72 ± 0.59        | 8.6       | 13.80–21.45               | 18.08 ± 0.42  | 11.8                  | 1.62–2.24  | 1.93 ± 0.03          | 8.9 prolate striate |
| 4   | N. sibirica        | Shelek    | 30.68–38.86  | 36.36 ± 0.46        | 6.3       | 15.18–21.99               | 19.36 ± 0.36  | 9.4                   | 1.64–2.15  | 1.89 ± 0.02          | 7.2 prolate striate |
| 5   | N. sibirica        | Balansor  | 36.11–43.33  | 40.24 ± 0.34        | 4.3       | 17.07–23.50               | 20.48 ± 0.30  | 7.5                   | 1.73–2.28  | 1.97 ± 0.02          | 5.8 prolate striate |
| 6   | N. sibirica        | Basshi    | 31.41–36.00  | 33.82 ± 0.32        | 4.8       | 21.10–27.08               | 25.81 ± 0.50  | 9.7                   | 1.10–1.51  | 1.32 ± 0.02          | 6.7 subprolate or prolate striate-perforate |
| 7   | N. sibirica        | Koktal    | 35.92–40.28  | 37.90 ± 0.24        | 3.2       | 19.88–23.73               | 21.89 ± 0.20  | 4.7                   | 1.58–1.82  | 1.78 ± 0.01          | 3.7 prolate striate |
| 8   | N. sibirica        | Taskarasu | 28.71–36.94  | 33.22 ± 0.46        | 6.9       | 22.10–24.58               | 23.05 ± 0.13  | 2.9                   | 1.28–1.65  | 1.44 ± 0.02          | 6.9 subprolate or prolate striate-perforate |
| 9   | N. sibirica        | Karatal   | 30.95–37.29  | 33.87 ± 0.36        | 5.4       | 23.23–26.17               | 24.37 ± 0.16  | 3.4                   | 1.26–1.53  | 1.39 ± 0.01          | 6.1 subprolate or prolate striate-perforate |
| 10  | N. sibirica        | Kurti     | 31.95–42.72  | 35.33 ± 0.55        | 7.8       | 17.24–23.39               | 20.79 ± 0.31  | 7.5                   | 1.53–1.87  | 1.70 ± 0.01          | 6.0 prolate striate |
| 11  | N. komarovii       | Balhash   | 23.38–29.50  | 27.15 ± 0.30        | 5.6       | 14.23–18.91               | 16.42 ± 0.30  | 9.1                   | 1.44–1.89  | 1.66 ± 0.02          | 8.0 prolate striate |
| 12  | N. schoberi        | Balhash   | 32.59–46.32  | 38.33 ± 0.79        | 10.0      | 18.18–28.00               | 22.92 ± 0.62  | 13.0                  | 1.40–2.08  | 1.69 ± 0.03          | 10.3 prolate striate |
| 13  | N. schoberi        | Sarozezk | 38.19–49.90  | 42.84 ± 0.62        | 7.2       | 22.28–32.59               | 27.84 ± 0.65  | 11.0                  | 1.24–2.05  | 1.57 ± 0.05          | 16.5 prolate striate |
| 14  | N. schoberi        | Basshi    | 29.73–47.37  | 37.46 ± 1.03        | 13.0      | 19.32–31.78               | 24.07 ± 0.58  | 12.0                  | 1.19–2.14  | 1.57 ± 0.04          | 13.8 prolate striate |
| 15  | N. schoberi        | Taskarasu | 39.04–43.89  | 41.39 ± 0.26        | 3.1       | 24.77–27.40               | 26.37 ± 0.14  | 2.7                   | 1.47–1.67  | 1.57 ± 0.01          | 3.1 prolate striate |
| 16  | N. schoberi        | Karatal   | 36.43–47.14  | 41.92 ± 0.69        | 8.3       | 23.78–31.13               | 28.46 ± 0.44  | 7.8                   | 1.29–1.64  | 1.48 ± 0.01          | 6.4 prolate striate |
| 17  | N. schoberi        | Kulunda   | 30.66–49.06  | 41.17 ± 1.01        | 12.3      | 16.79–29.72               | 24.59 ± 0.77  | 15.6                  | 1.26–2.19  | 1.70 ± 0.04          | 13.1 prolate striate |
| 18  | N. schoberi        | Malinovec | 35.67–50.59  | 42.66 ± 0.90        | 10.5      | 22.58–35.89               | 26.46 ± 0.71  | 13.4                  | 1.30–2.16  | 1.63 ± 0.04          | 14.5 prolate striate |
| 19  | N. pamirica        | Shaimak   | 30.13–36.07  | 32.29 ± 0.32        | 4.9       | 24.87–30.62               | 26.50 ± 0.28  | 5.3                   | 1.18–1.27  | 1.22 ± 0.005         | 2.0 subprolate striate |
| 20  | N. tangutorum      | Type      | 37.70–46.81  | 42.72 ± 0.47        | 5.6       | 19.16–23.30               | 21.04 ± 0.19  | 4.5                   | 1.74–2.20  | 2.02 ± 0.02          | 4.8 prolate or perprolate striate |
| 21  | N. praevisa        | Type      | 34.14–40.01  | 37.55 ± 0.28        | 3.7       | 16.64–21.40               | 19.18 ± 0.23  | 6.2                   | 1.75–2.37  | 1.96 ± 0.02          | 7.4 prolate or perprolate striate |

Note: see Table 2.
Table 2. Vaucher specimens of *Nitraria* (Nitrariaceae).

| No. | Taxon       | Specimen | Locality                                                                 | Date          | Herbarium, Specimen Number |
|-----|-------------|----------|--------------------------------------------------------------------------|---------------|-----------------------------|
| 1   | *N. sibirica* | Balhash  | Republic of Kazakhstan, Almaty Region, on the shore of Lake Balkhash, sandy desert | 31 May 2016   | NSK3001248                  |
| 2   | *N. sibirica* | Kosh-Agach | Russia, Republic of Altay, Kosh-Agachskiy District, vicinity of Kosh-Agach village | 6 July 2018   | NSK3001271                  |
| 3   | *N. sibirica* | Dauria   | Russia, Transbaikal, Borzinsky District, Daursky Reserve, on the shore of Lake Zun-Torey | 8 July 2012   | NSK3001266                  |
| 4   | *N. sibirica* | Shelek   | Republic of Kazakhstan, Almaty Region, vicinity of Shelek village         | 26 May 2016   | NSK3001242                  |
| 5   | *N. sibirica* | Balansor | Russia, Altai Krai, Uglovskiy District, on the shore of Lake Balansor     | 1 June 2011   | NSK3001280                  |
| 6   | *N. sibirica* | Basshi   | Republic of Kazakhstan, Almaty Region, vicinity of Bashshi village        | 25 July 2016  | NSK3001249                  |
| 7   | *N. sibirica* | Koktal   | Republic of Kazakhstan, Almaty Region, vicinity of Koktal village         | 25 May 2016   | NSK3001236                  |
| 8   | *N. sibirica* | Taskarasu | Republic of Kazakhstan, Almaty Region, vicinity of Taskarasu village      | 26 May 2016   | NSK3001244                  |
| 9   | *N. sibirica* | Karatal  | Republic of Kazakhstan, Almaty Region, Karatalskiy District, vicinity of Ushtobe city, on the terrace of the Karatal River | 29 May 2016   | NSK3000922                  |
| 10  | *N. sibirica* | Kurti    | Republic of Kazakhstan, Almaty Region, Iliyskiy District, north of Kurty village, on the bank of the Kurty River | 27 May 2016   | NSK3001241                  |
| 11  | *N. konaroffii* | Balhash | Republic of Kazakhstan, Almaty Region, on the shore of Lake Balkhash, sandy desert | 31 May 2016   | NSK3000920                  |
| 12  | *N. schoberi* | Balhash  | Republic of Kazakhstan, Almaty Region, on the shore of Lake Balkhash, sandy desert | 31 May 2016   | NSK3000948                  |
| 13  | *N. schoberi* | Sariozek | Republic of Kazakhstan, Almaty Region, 30 km north of Saroysky village   | 25 May 2016   | NSK3000947                  |
| 14  | *N. schoberi* | Basshi   | Republic of Kazakhstan, Almaty Region, vicinity of Bashshi village        | 25 May 2016   | NSK3000982                  |
| 15  | *N. schoberi* | Taskarasu | Republic of Kazakhstan, Almaty Region, vicinity of Taskarasu village      | 26 May 2016   | NSK3000954                  |
| 16  | *N. schoberi* | Karatal  | Republic of Kazakhstan, Almaty Region, Karatalskiy District, vicinity of Ushtobe town, on the terrace of the river Karatal | 29 May 2016   | NSK3000952                  |
| 17  | *N. schoberi* | Kulunda  | Russia, Altai Krai, Slavgorodskiy District, on the shore of Lake Kulundinsko 2 | 2 June 2011    | NSK3000975                  |
| 18  | *N. schoberi* | Malinove  | Russia, Altai Krai, Mikhailovskiy District, on the shore of Lake Malinovoe  | 1 June 2011  | NSK3000971                  |
| 19  | *N. pamirica* | Shaimak  | Republic of Tajikistan, Gorno-Badakhshan Autonomous Region, Eastern Pamir, on the cliff of the Dilja River | 10 August 2014 | NSK3001238                  |
| 20  | *N. tangutorum* | Type    | Zaidan orientalis, in vicinity Barun-zsasak 1 | 17 June 1901  | LE01025061                 |
| 21  | *N. praecissa* | Type    | China, prov. Ganju 67 rm septentroniem versus ab Lanjchshou | 29 June 1957  | LE01043513                 |
Figure 1. LM micrographs of *Nitraria* pollen: (a) equatorial view for *N. komarovii*; (b) polar view for *N. pamirica*; (c) equatorial view in the mesocolpus area of *N. tangutorum*; (d1) equatorial view in the mesocolpus area of *N. praevisa*; (d2) semipolar view for *N. praevisa*; (e1–e9) *N. sibirica* specimens from (e1) Balansor, (e2) Balhash, (e3) Basshi, (e4) Dauria, (e5,e6) Kosh-Agach, (e7) Shelek, and (e8,e9) Kurti; (f1–f5) *N. schoberi* specimens from (f1) Balhash, (f2) Karatal, (f3) Malinovoe, (f4,f5) Sariozek, and (f6) Taskarasu. Bar: 10 μm.
Figure 2. SEM micrographs of *Nitraria* pollen: (a1) equatorial view showing a colpus, (a2) equatorial view in the mesocolpus area, (a3) polar view, and (a4) details of the exine surface;
N. komarovi: (b1) equatorial view showing a colpus, (b2) equatorial view in the mesocolpus area, (b3) polar view, and (b4) details of the exine surface; N. schoberi: (c1) equatorial view showing a colpus and porus, (c2) equatorial view in the mesocolpus area, (c3) polar view, and (c4) details of the exine surface; N. pamirica: (d1) equatorial view showing a colpus, (d2) equatorial view in the mesocolpus area, (d3) equatorial view showing a colpus and porus, and (d4) details of the exine surface; N. tangutorum: (e1) equatorial view showing a colpus, (e2) equatorial view in the mesocolpus area, (e3) semipolar view, and (e4) details of the exine surface; N. praevisa: (f1) equatorial view showing a colpus, (f2) equatorial view in the mesocolpus area, (f3) polar view, and (f4) details of the exine surface.

2.1. Morphological Characteristics of the Genus Nitraria

According to Erdtman’s [29] pollen size classification, pollen grains of the Nitraria species under study are medium-sized (25–50 µm), and only in N. schoberi specimens were there pollen grains up to 50.59 µm (classified as large). Pollen is monad, isopolar, and radially symmetric. The pollen shape is circular, pseudo-hexagonal, hexagonal, or triangular convex in polar view and subprolate to perprolate in equatorial view. The aperture is tricolporate, and colpi are long and narrow, usually constricted at the equator and have costae colpi and a fastigium. The endoapertures are formed by lalongate pores, which are elliptical to rhomboidal in shape. The exine is tectate, with the nexine thicker than the sexine; the surface is striate to striate–perforate.

2.1.1. Morphological Characteristics of N. sibirica

Pollen grains of N. sibirica are prolate in equatorial view and triangular convex or pseudo-hexagonal, circular in polar view and monad, isopolar, and radially symmetric (Figures 1(e1–e9) and 2(a1–a3)), size: length: 27.60–44.72 µm; width: 13.80–24.71 µm, with an average ratio of the polar axis to equatorial axis (P/E) of 1.70–1.97 (Table 1).

Some specimens of N. sibirica have pollen grains that are subprolate or prolate in equatorial view and circular in polar view and have a length of 28.71–37.29 µm, width of 21.10–27.08 µm, with an average P/E ratio of 1.10–1.53.

Aperture: tricolporate; ectoaperture: colpus, almost as long as the polar axis, open (4/5 of polar axis), straight, narrow, occasionally constricted at equator with ends acute; polar area asymmetric. Margin observed in polar view, costae colpi and fastigium conspicuous in equatorial view. Endoaperture: porus, conspicuous, lalongate, elliptic to rhomboidal in shape. Exine: tectate; exine slightly thicker in polar areas in relation to equatorial region; nexine thicker than sexine. Exine ornamentation is striate, no perforations observed. Striae relatively loosely packed and short, running fairly parallel to the polar axis near the colpus and in the mesocolpus area (Figure 2(a4)).

2.1.2. Morphological Characteristics of N. komarovii

Pollen grains of N. komarovii are prolate in equatorial view and triangular convex to pseudohexagonal in polar view, and they are monad, isopolar, and radially symmetric (Figures 1a and 2(b1–b3)), medium in size: length: 27.15 (23.38–29.50) µm; width: 16.42 (14.23–18.91) µm, with an average P/E ratio of 1.66 (Table 1).

Aperture: tricolporate; ectoaperture: colpus, almost as long as the polar axis, open (2/3 of polar axis), straight, narrow, occasionally constricted at equator with ends acute; polar area asymmetric. Margin observed in polar view, costae colpi and fastigium conspicuous in equatorial view. Endoaperture: porus, conspicuous, lalongate, elliptic to rhomboidal in shape. Exine: tectate; exine slightly thicker in polar areas in relation to equatorial region; nexine thicker than sexine. Exine ornamentation is striate, no perforations observed (Figure 2(b4)). Striae relatively tight and short running parallel to the polar axis near the colpus while running slightly counterclockwise in the mesocolpus area, eventually forming a spiral “cluster” in one area close to a pole or running almost parallel.
2.1.3. Morphological Characteristics of *N. schoberi*

Pollen grains of *N. schoberi* are prolate in equatorial view and triangular convex to hexagonal in polar view, monad, isopolar, radially symmetric (Figures 1(f1–f5) and 2(c1–c3)), medium in size: length: 32.59–50.59 µm; width: 14.23–35.89 µm, with an average P/E ratio of 1.57–1.70 (Table 1).

Aperture: tricolporate. Ectoaperture: colpus, almost as long as the polar axis, open (4/5 of polar axis), straight, narrow, occasionally constricted at equator with ends acute; polar area asymmetric. Margin observed in polar view, costae colpi and fastigium conspicuous in equatorial view. Endoaperture: porus, conspicuous, lalongate, elliptic to rhomboidal in shape. Exine: tectate; exine slightly thicker in polar areas in relation to equatorial region; nexine thicker than sexine. Exine ornamentation is striate, no perforations observed. Striae relatively loosely packed and short, running fairly parallel to the polar axis near the colpus and in the mesocolpus area (Figure 2(c4)).

2.1.4. Morphological Characteristics of *N. pamirica*

Pollen grains of *N. pamirica* are subprolate in equatorial view and circular to hexagonal in polar view, and monad, isopolar, and radially symmetric (Figures 1b and 2(d1–d3)), medium in size: length: 32.29 (30.13–36.07) µm; width: 26.50 (24.87–30.62) µm, on average with a P/E ratio of 1.22 (Table 1).

Aperture: tricolporate. Ectoaperture: colpus, almost as long as the polar axis, open (2/3 of polar axis), straight, narrow, occasionally constricted at equator with ends acute; polar area asymmetric. Margin observed in polar view, costae colpi and fastigium conspicuous in equatorial view. Endoaperture: porus, conspicuous, lalongate, elliptic to rhomboidal in shape. Exine: tectate; exine slightly thicker in polar areas in relation to equatorial region; nexine thicker than sexine. Exine ornamentation is striate, no perforations observed. Striae relatively tight and short running parallel to the polar axis near the colpus (Figure 2(d4)).

2.1.5. Morphological Characteristics of *N. tangutorum*

Pollen grains of *N. tangutorum* are perprolate in equatorial view and triangular convex to circular in polar view and monad, isopolar, and radially symmetric (Figures 1c and 2(e1–e3)), medium in size: length: 42.72 (37.70–46.81) µm; width: 21.04 (19.16–23.30) µm, on average with a P/E ratio of 2.02 (Table 1).

Aperture: tricolporate. Ectoaperture: colpus, almost as long as the polar axis, open (4/5 of polar axis), straight, narrow, occasionally constricted at equator with ends acute; polar area asymmetric. Margin observed in polar view, costae colpi and fastigium conspicuous in equatorial view. Endoaperture: porus, conspicuous, lalongate, elliptic to rhomboidal in shape. Exine: tectate; exine slightly thicker in polar areas in relation to equatorial region; nexine thicker than sexine. Exine ornamentation is striate, no perforations observed (Figure 2(e4)). Striae relatively tight and short running parallel to the polar axis near the colpus while running slightly counterclockwise in the mesocolpus area, eventually forming a spiral “cluster” in one area close to a pole or running almost parallel.

2.1.6. Morphological Characteristics of *N. praevisa*

Pollen grains of *N. praevisa* are perprolate in equatorial view and triangular convex to hexagonal in polar view, and they are monad, isopolar, and radially symmetric (Figures 1(d1,d2) and 2(f1–f3)), medium in size: length: 37.55 (34.14–40.01) µm; width: 19.18 (16.64–21.40) µm, with an average P/E ratio of 1.96 (Table 1).

Aperture: tricolporate. Ectoaperture: colpus, almost as long as the polar axis, open (4/5 of polar axis), straight, narrow, occasionally constricted at equator with ends acute; polar area asymmetric. Margin observed in polar view, costae colpi and fastigium conspicuous in equatorial view. Endoaperture: porus, conspicuous, lalongate, elliptic to rhomboidal in shape. Exine: tectate; exine slightly thicker in polar areas in relation to equatorial region; nexine thicker than sexine. Exine ornamentation is striate, no perforations observed (Figure 2(f4)). Striae relatively tight and short running parallel to the polar axis near the
3. Discussion

Overall, for all the analyzed taxa of Nitraria, a characteristic feature is radially symmetric, monad, isopolar pollen grains. The pollen shape is circular, pseudo-hexagonal, hexagonal, or triangular convex in polar view and perprolate to subprolate in equatorial view. The aperture is tricolporate, and colpi are long and narrow. In the comparison of average lengths of the polar and equatorial axes of Nitraria pollen grains, it turned out that the average length of the polar axis in N. tangutorum, and in most specimens of N. schoberi (Sariozek, Taskarasu, Karatal, Kulunda, and Malinovoe), it is greater than that in the other species, whereas the average equatorial axis length in N. komarovi, N. praevisa, and N. sibirica is less than that in N. schoberi and N. pamirica (Table 1). The polar axis and equatorial axis of pollen grains in the genus Nitraria vary continuously and overlap in their ranges. The smallest pollen grains were found in N. komarovi (27.15 × 16.42 µm), and the largest ones were found in specimens of N. schoberi (42.87 × 27.84 µm).

A comparison and analysis of P/E ratios of pollen grains within the genus Nitraria showed that the highest P/E values belong to N. tangutorum and N. praevisa (2.02 and 1.96, respectively), and indeed, these species have a perprolate pollen shape. In N. komarovi and specimens of N. schoberi, the P/E ratio is in the range of 1.57–1.70. The lowest P/E value was detected in N. pamirica pollen grains (1.22), which possess a subprolate shape (Figure 2(d1–d3)). Most N. sibirica specimens (Balhash, Kosh-Aghach, Dauria, Shelek, Balansor, Kurti, and Koktal) have P/E in the range of 1.70–1.97. By contrast, specimens of N. sibirica (Taskarasu, Karatal, and Basshi) have much lower P/E, 1.32–1.44, and pollen grains have a subprolate or prolate shape (Figure 3(d1,d2,h1,h2,i1,i2)).

Principal component analysis (PCA) of the main morphological traits of pollen grains in the Nitraria species uncovered an obvious clustering of the specimens (Figure 4). Two principal components, PC1 and PC2, together accounted for 98.86% of the total variance. N. komarovi (specimen No. 11) is an outlier, whereas N. pamirica (specimen No. 19) and N. sibirica specimens Basshi, Taskarasu, and Karatal (No. 6, 8, and 9, respectively) stand out as a small group. The two remaining clusters clearly represent a group of N. schoberi specimens (Balhash, Sariozek, Basshi, Taskarasu, Karatal, and Malinovoe) and a group consisting of N. tangutorum, N. praevisa, and specimens of N. sibirica (Balhash, Kosh-Aghach, Dauria, Shelek, Balansor, Kurti, and Koktal).

In addition, N. sibirica specimens Basshi, Taskarasu, and Karatal were found to have a longer equatorial axis and a shorter polar axis than do specimens of N. sibirica (Balhash, Kosh-Aghach, Dauria, Shelek, Balansor, Kurti, and Koktal; Table 1). Differences of N. sibirica specimens Basshi, Taskarasu, and Karatal from the other specimens of N. sibirica lie in pollen grain ornamentation. In these specimens, exine ornamentation is striate and perforate. Striae are relatively loose, packed in the mesocolpia, and short (Figure 3k,l). Previously, we have documented differences between the populations of N. sibirica from Basshi and Taskarasu in many morphological traits of vegetative and generative organs, bush habitus [30], the profile and levels of phenolic compounds [31,32], and in the analysis of the ITS2 region of nuclear ribosomal DNA [33,34]. The above findings support the taxonomic separation of these populations.

Our results on the type and shape of pollen grains do not contradict earlier descriptions [27,28]. There are minor differences in the size of pollen grains, in the width of furrows, and in the size of pores; this can be explained by the different environmental conditions of the plant specimens in question.
Figure 3. SEM micrographs of *N. sibirica* pollen. Collection sites of the photographed specimens: (a1,a2) Dauria; (b1,b2) Shelek; (c1,c2) Balansor; (d1,d2) Karatal; (e1,e2) Balhash; (f1,f2) Kurti; (g1,g2) Kosh-Agach; (h1,h2) Taskarasu; (i1,i2) Basshi; (j1,j2) Koktal; (k) Kosh-Agach, details of the exine surface; and (l) Basshi, details of the exine surface.
When comparing pollen grains in a single anther, we noticed that in species *N. komarovii*, *N. tangutorum*, *N. praevisa*, and *N. pamirica* and in all specimens of *N. sibirica*, pollen grains have an almost stable shape and size (Figures 3(a1,b1,c1,d1,e1,f1,g1,h1,i1,j1) and 5a–e). In *N. schoberi* in all populations, pollen grains of various shapes can be found (Figure 5(f1–f7)).

For instance, the rounded shape occurred more often in populations Balhash, Sarozzek, Karatal, Kulunda, and Malinovoe (Figures 5(f1,f2) and 6(a3,b1,e2,f4)), and the conical shape occurred more often in populations Kulunda, Sarozzek, and Taskarasu (Figure 6(a2,e2,f4)).

Overall, bipolar, tri-furrowed pollen grains are characteristic of *N. schoberi*. In the work of Sladkov [35], the presence of pollen grains deviating from typical ones was documented for this species. In eight populations (the Lower Volga region, the Crimea (town of Koktebel), the foothills of Dagestan, the coast of the Aral Sea, the western slope of the Mugodzhar Mountains, the southeastern shore of Lake Kainar-Kul, Kazalinsk, and Kara-Ketken), he registered four-pole four-furrowed and four-pole six-furrowed pollen grains. Along with these two types of pollen grains, that author noticed clearly deformed pollen grains with underdeveloped and irregularly oriented furrows. Agababian and Tumanyan [27], having researched the pollen polymorphism of *N. schoberi* from various geographical locations (Semipalatinsk region (Karabelgu), Mangyshlak peninsula (Aktobe), Inner Mongolia (Bayan-Khoto), and Turchay region (Naurzum Karasu mouth)), did not find any abnormal pollen grains. An exception was a specimen of *N. schoberi* var. *caspica* Pall. from Sarepta: it had some anomalous pollen grains.
Figure 5. SEM micrographs of *Nitraria* pollen. (a) *N. sibirica*; (b) *N. komarovi*; (c) *N. pamirica*; (d) *N. tangutorum*; (e) *N. praevisa*; (f1–f7) *N. shoberi* from (f1) Balhash, (f2) Kulunda, (f3) Malinovoe, (f4) Karatal, (f5) Basshi, (f6) Taskarasu, and (f7) Sariozek.
Figure 6. Deformed pollen grains of *N. schoberi* from (a1–a3) Kulunda, (b) Malinovoe, (c) Karatal, (d1,d2) Basshi, (e1,e2) Sariozek, (f1,f2) Taskarasu, and (g) Balhash.

We also detected four-pole four-furrowed (Figure 6(d1,e1)) and four-pole six-furrowed (Figure 6(c1,d2,f1,g1)) pollen grains in all specimens of *N. schoberi*. The presence of such pollen grains only in populations of *N. schoberi* can serve as a diagnostic criterion for this species and requires deeper investigation into the reasons for their deformation.
4. Materials and Methods

4.1. Plant Material

We studied six species of *Nitraria* that occur in Russia (Altai Krai, Republic of Altai, Transbaikal), Kazakhstan, and Tajikistan (Figure 7). Specimens of species *N. sibirica* (10 locations), *N. schoberi* (7 locations), *N. komarovii* Iljin & Lava ex Bobrov, and *N. pamirica* L. Vassil. were collected in natural settings in 2011–2018. The collectors were E.V. Banaev and M.A. Tomoshevich. Herbarium specimens were deposited in a collection at the Central Siberian Botanical Garden, the Siberian Branch of the Russian Academy of Sciences (Herbarium of the Laboratory of Dendrology, NSK Collection, Digital Herbarium; http://herb.csbg.nsc.ru:8081 accessed on 8 September 2022). *N. tanguorum* Bobr. and *N. praevisa* were acquired as herbarium specimens that belong to original material from the Komarov Botanical Institute of the Russian Academy of Sciences (LE). The voucher information about all the specimens is shown in Table 2.

![Figure 7](image_url)

**Figure 7.** The map of sampling sites of species from the genus *Nitraria*. White color, *N. sibirica*; red color, *N. schoberi*; yellow color, *N. komarovii*; and blue color, *N. pamirica*.

4.2. LM and SEM

Air-dried pollen grains of *Nitraria* species were processed by standard acetolysis [36] and were kept in glycerol. For LM, pollen grains were analyzed by means of a Carl Zeiss Axioskop 40 microscope equipped with a digital camera AxioCam MRc 5 and the AxioVision 4.8 software (Carl Zeiss, Göttingen, Germany).

For SEM examination, the air-dried pollen grains were transferred directly to a stub covered with double-sided transparent tape to prevent pollen dispersion due to pressure. The stub was inspected under a binocular microscope to ensure the pollen was distributed evenly. Pollen grains that clumped together were separated by lightly brushing with a cotton bud, and loose pollen grains were removed by a stream of dry air. The studs were coated with gold in a Mini SC 7620 sputter coater (Quorum Technologies, West Sussex, UK) and photographed under an EVO MA10 (Carl Zeiss, Germany) scanning electron microscope at 20 kV.

During this analysis, morphological traits of pollen grains were examined, including the polar axis, equatorial axis, pollen grain shape, and surface ornamentation. To be precise, the following traits were characterized: pollen size and shape (polar axis (P), equatorial axis (E), and the P/E ratio), polarity, symmetry, aperture, and exine ornamentation.
4.3. Data Analysis

The P and E of pollen grains were determined in 25 grains randomly chosen from 5 slides of 35 each specimen by LM. All data were analyzed to calculate the mean (X), standard deviation (S), standard error (Sx), a confidence interval (95% CI), and the coefficient of variation (CV, %).

The palynological terminology is borrowed from Punt et al. [37], Hesse et al. [38], and Halbritter et al. [39]. The pollen shape class, based on the P/E ratio, was identified using Erdtman’s system [29].

Genotypes were clustered into similarity groups by PCA. All statistical analyses were performed in the STATISTICA 6.0 software (TIBCO Software Inc., Palo Alto, CA, USA).

5. Conclusions

In this palynomorphological study, we demonstrated that the morphological traits of pollen grains are important for the systematics of the genus Nitraria. Overall, for all the analyzed taxa of Nitraria, a characteristic feature is radially symmetric, monad, isopolar pollen grains. The pollen shape is circular, pseudo-hexagonal, hexagonal, or triangular convex in polar view and perprolate to subprolate in equatorial view. The aperture is tricolporate, and colpi are long and narrow. It was established that N. komarovii possesses the smallest pollen grains, and the largest ones belong to specimens of N. schoberi. N. langutorum is characterized by the perprolate pollen shape, N. praevisa, N. komarovii, and N. schoberi are characterized by prolate, and N. pamirica is characterized by the subprolate shape. An intraspecific differentiation of N. sibirica was noted. In a population of N. sibirica (Basshi), pollen grains of a subprolate shape were detected, whereas the prolate pollen shape is characteristic of other populations under study. Additionally, in N. sibirica populations Basshi, Karatal, and Taskarasu, exine ornamentation is striate–perforate, whereas the equatorial axis is longer and the polar axis is shorter as compared to specimens of N. sibirica (Balhash, Kosh-Agach, Dauria, Shelek, Balansor, Kurti, and Koktal). A comparison of pollen grains in one anther of N. schoberi in all populations revealed the presence of different shapes of pollen grains (rounded, conical, four-pole four-furrowed, and four-pole six-furrowed). The results of the current study expand the palynomorphological data for Nitraria and will also contribute to future phylogenetic and taxonomic studies in this genus.

Author Contributions: M.T. and E.B. collected the pollen during expeditions; M.T., E.B., S.K. and A.E. participated in study design, sampling, and data processing; M.T. and A.E. participated in the sampling and performed laboratory analyses (microscopy); M.T. and E.B. identified the problem, interpreted the results, and wrote the manuscript; S.K. and A.E. edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Higher Education of the Russian Federation program of scientific research “Theoretical and applied aspects of studying gene pools of natural plant populations and conservation of plant diversity ‘outside the typical environment’ (ex situ)” (AAAA-A21-121011290027-6).

Data Availability Statement: Raw data are available upon request.

Acknowledgments: During the preparation of this publication, materials of bioresource scientific collections of the CSBG SB RAS “Collections of living plants indoors and outdoors,” unique scientific unit USU_440534, and “Herbarium of higher plants, lichens and fungi (NS, NSK)” USU-4450537 were used. The authors are grateful to Nikolai A. Shevchuk for translating the manuscript into English.

Conflicts of Interest: The authors declare no conflict of interest.

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