



# Pollen morphology of *Degenia velebitica* (Degen) Hayek and *Sibiraea altaiensis* (Laxm.) C. K. Schneid. subsp. *croatica* Degen – rare Croatian endemic plants from Velebit Mountains

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## Abstract

**Background and Purpose:** Pollen can, as a means of genetic resource, contribute to conservation of endemic plants. Therefore we started with palynological study of endemic plants from refuges of the Alpine-Dinaric region and here present preliminary results on pollen morphology of two most famous representatives of Croatian endemic plants from Velebit Mts, *Degenia velebitica* and *Sibiraea altaiensis* subsp. *croatica*.

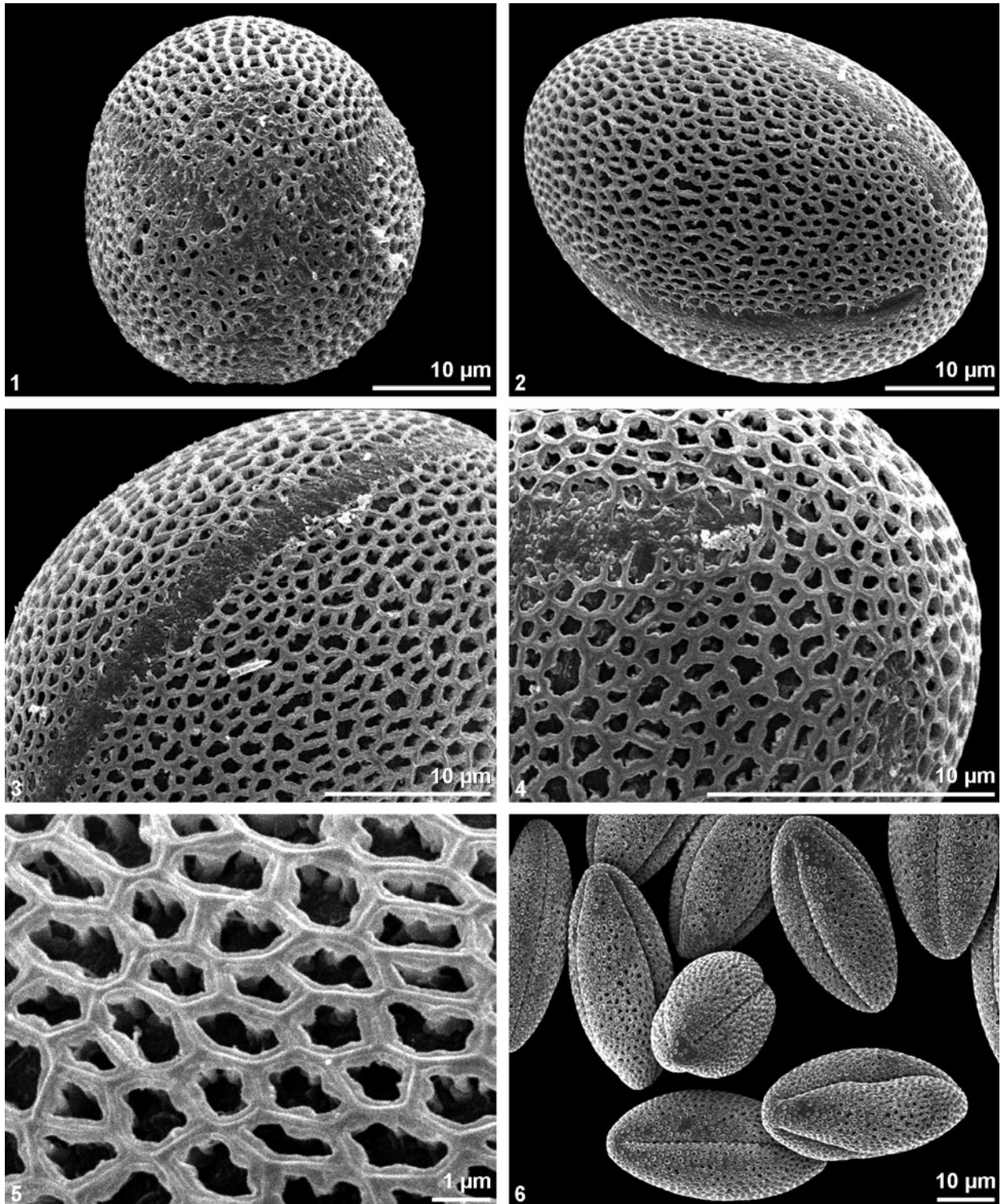
**Methods:** Pollen samples from field collections were examined by the JEOL JSM-T 300 Scanning Electron Microscope in dry and hydrated state. For hydrated state, pollen grains were briefly rehydrated in water, dehydrated in acidified 2, 2-dimethoxypropane (DMP) and critical point dried in CO<sub>2</sub>. Dry pollen was mounted on stubs without further preparation. Both dry and critical point dried material was mounted on stubs and sputter coated with gold.

**Results:** Pollen grains of *Degenia velebitica* are tricolpate, prolate and about 35 – 40 μm in diameter (longest axis). The colpi are extended and quite narrow. The exine ornamentation is reticulate homobrochate, lumina width is small (less than 2 μm in diameter). Dry pollen is prolate with infolded apertures. Pollen grains of *Sibiraea altaiensis* subsp. *croatica* are tricolporate, spheroidal to slightly oblate (hydrated condition) and about 25 μm in diameter. The apertures are quite smooth. Exine ornamentation is striate perforate consisting of coarse striae with perforations in the grooves. Dry pollen is prolate with infolded apertures.

**Conclusion:** Evidence from pollen morphology of both taxa indicate a possibility of closer relationships with their relatives in the past. Despite this, both taxa developed their own palynological uniqueness and deserved conservation of their pollen samples.

## INTRODUCTION

Croatia takes the third place in Europe in floristic richness with 5599 taxa (species and subspecies) of vascular flora (cf. 1, 2). 357 endemic taxa were registered in various categories of endemic plants which are of the largest interest to Croatia and its diversity, but also to diversity of the whole Alpine-Dinaric region. This is an area with many



**Figure 1.1.-6.** *Degenia velebitica*, pollen grains, SEM. 1.1-5. in hydrated (turgescient) state: 1.1. pollen grain in polar view; 1.2. pollen grain in equatorial view; 1.3. aperture (colpus); 1.4. polar area; 1.5. exine surface, homobrochate reticulum; 1.6. dry pollen, apertures are infolded.

postglacial refuges, rich in geographically separated and isolated populations of related plant taxa, often taxonomically distinguished as separate species or subspecies, with an endemic character.

Pollen can, as a means of genetic resource, contribute to conservation approaches through taxonomic studies and collection of pollen samples and their preservation (3). As pollen characters (aperture type, pollen wall sculptur-

ing, pollen shape, etc.) are taxonomically and phylogenetically important, they could point to relationships between related and/or separate taxonomic groups. Special interests concern pollen features of the mentioned endemic taxa from refuges of the Alpine-Dinaric region, especially those with IUCN threatened status, for which pollen features can contribute much information about their origin, variability and relationships with other groups. Thus palynological information, together with results of other phylogenetic methods (e.g. molecular), could give us guidelines for possible biological conservation and protection of endemic plants at both national and international levels.

Therefore we started with palynological studies of Croatian endemic plants and, after achieving first results (4), we decided to start with complex palynological study, in a tendency to extend the research to the whole refugial Alpine-Dinaric region, known as an area rich with endemic plants.

Here we present results of the first preliminary study on pollen morphology of the two most famous representatives of Croatian endemic plants from Velebit Mts, *Degenia velebitica* (Degen) Hayek and *Sibiraea altaiensis* (Laxm.) C. K. Schneid. subsp. *croatica* Degen, as part of preparations for the conservation of targeted endemic and rare plants in Croatia (1, 5).

Species *Degenia velebitica* (Degen) Hayek (Syn. *Lesquerella velebitica* Degen, *Alyssum velebiticum* Degen, *Vesicaria velebitica* Degen) is a well-known monotypic genus from the family Brassicaceae (6), whose natural area of distribution is on only several localities on the Velebit mountains (7) and therefore it is exclusively stenoendemic to Croatia. It grows in a form of small, dense plant on limestone screes and rock-crevice habitats. Data for *D. velebitica* available until our study were about its morphology and anatomy (8, 9), seed germination (10), micropropagation (11) and cytogenetics (12, 13). *D. velebitica* has been classified as a critically endangered taxon (EN) on Croatian Red List (1). It is also included as vulnerable on European (14) and World Red Lists (15). According to Croatian Nature Conservation Act *D. velebitica* is protected in all natural localities and by new Regulations on Collecting Plants for the Purpose of Manufacturing, Trade and Other Manipulation (16). Indirectly, Regulations on Internal Legality protects *D. velebitica* within the area of the Velebit Nature Park.

Taxon *Sibiraea altaiensis* (Laxm.) C. K. Schneid. subsp. *croatica* Degen (Syn. *Sibiraea croatica* Degen, *Sibiraea altaiensis* (Laxm.) C.K. Schneider var. *croatica* (Degen) G. Beck), is a rare and endemic bushy species spread only in Croatia on the Velebit Mts (within the *Seslerio-Ostryetum* Horv. & H-ić ass. and at the edges of the littoral common beech) and in Bosnia and Herzegovina on Čvrtnica and Čabulja Mt (on limestone on open rocky surfaces, screes and high mountain pastures).

In the above mentioned regions it represents a tertiary relic, although its taxonomic position as a subspecies has not been entirely defined yet. Actually, its closest relative,

*Sibiraea altaiensis* (Laxm.) C.K. Schneider, grows in Central Asia, 5000 km away from Croatian and Herzegovinian plants, but from both morphological (17) and molecular-genetic (18) studies they seem to be very similar. In its natural sites, *S. altaiensis* subsp. *croatica* was protected in 1964 and is in nearly threatened (NT category) according to a new Croatian Red List of Vascular Plants (2). It is indirectly protected by Regulations on Collecting Plants for the Purpose of Manufacturing, Trade and Other Manipulation, as well as Regulation on Internal Legality inside the area of Paklenica National Park (16). *S. altaiensis* subsp. *croatica* is also a protected endemic species in Bosnia and Herzegovina, but hitherto without exact protection measures (18).

According to present studies both taxa, *Degenia velebitica* and *Sibiraea altaiensis* subsp. *croatica*, deserve active conservation approaches to protect their ancient sites as well as the plants themselves. In that sense, pollen features will complete the data about their biology known so far.

## MATERIALS AND METHODS

We examined pollen grains of two taxa, representatives of the Croatian endemic plants (pollen samples were obtained from field collections, during the vegetation season 2003), whose nomenclature is as follows (2):

1. *Sibiraea altaiensis* subsp. *croatica* (collected in June 2003; Velebit Mts, Velinac; co-ordinate  $x = 5503170.88$ ,  $y = 4939455.44$ ; voucher specimen ZA 388),

2. *Degenia velebitica* (collected in April 2003; Velebit Mts, Šugarska Duliba; co-ordinate  $x = 5519892.62$ ,  $y = 4923875.29$ ; voucher specimen ZA 392).

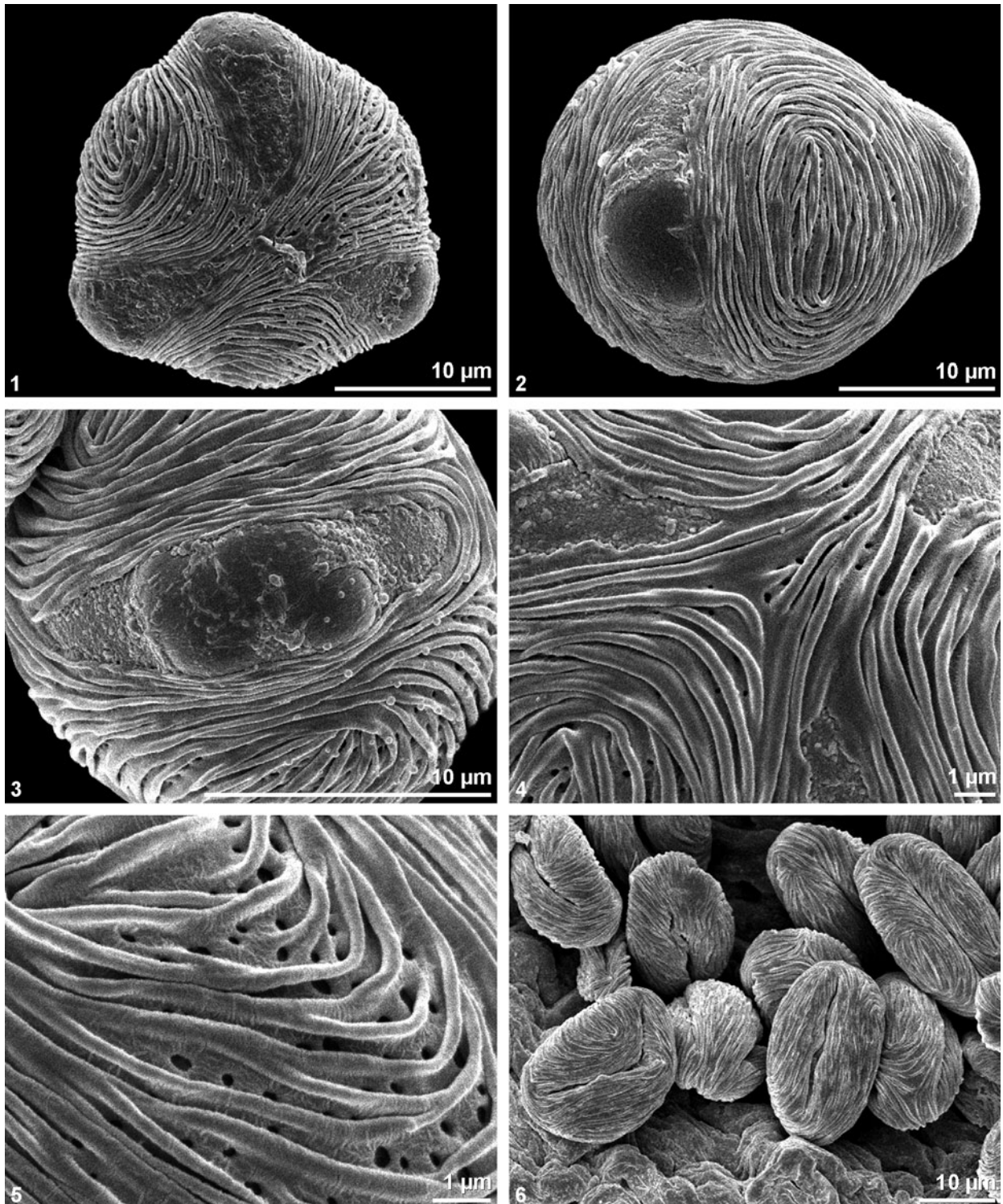
Fresh, mature pollen grains were collected at the beginning of anthesis and stored dry. To show the hydrated (turgescence) state in SEM, pollen grains were briefly rehydrated in water, dehydrated in acidified 2, 2-dimethoxypropane (DMP) and critical point dried in CO<sub>2</sub>. Air dried pollen was mounted on stubs without further preparation. Both untreated air dried and critical point dried material was mounted on stubs and sputter coated with gold (according to 19). The observations were made with the JEOL JSM-T 300 scanning electron microscope at the Department of Palynology and Structural Botany of the University of Vienna. Descriptions follow (20).

## RESULTS AND DISCUSSION

Pollen grains of *Degenia velebitica* are tricolpate (Figs. 1.1-6). In hydrated (turgescence) condition, pollen grains are prolate and about 35 – 40  $\mu\text{m}$  in diameter (longest axis) (Figs. 1.1, 1.2). The colpi are extended and quite narrow (Figs. 1.1-1.3). The exine ornamentation is reticulate homobrochate, lumina width is small (less than 2  $\mu\text{m}$  in diameter) (Figs. 1.4, 1.5). Dry (untreated) pollen is also prolate with infolded apertures (Fig. 1.6).

Details of palynology and pollen morphology of some other taxa from the family Brassicaceae, related with *Degenia*, are known (cf. 21, 22, 23, 24); the most interesting





**Figure 2.1-6.** *Sibiraea altaiensis* subsp. *croatica*, pollen grains, SEM. 2.1-5. in hydrated (turgescit) state: 2.1. pollen grain in polar view; 2.2. pollen grain in equatorial view; 2.3. aperture (colporus); 2.4. polar area; 2.5., exine surface, coarse striae and perforations; 2.6. dry pollen, apertures are infolded.

are the pollen grains of *Lesquerella* (25), within which Degen (26) firstly described plants from Velebit Mts. Comparison of *Degenia* and *Lesquerella* pollen morphology showed that both genera have similar pollen grains in

shape (aperture type is three-colpate, colpi are very long and strait, pollen wall sculpturing is reticulate, with small lumina width) and size, which can indicate their possible phylogenetical connections. However, pollen morphology

of the family Brassicaceae is similar in most Genera investigated so far (e.g. 21, 22, 23, 24) and further studies are necessary for better conclusions and understanding of possible earlier relationships between genus *Lesquerella*, distributed in both Americas (27), and *Degenia*.

Pollen grains of *Sibiraea altaiensis* subsp. *croatica* are tricolporate (Figs. 2.1-6). In hydrated (turgescens) condition, pollen grains are spheroidal to slightly oblate and about 25µm in diameter (Figs. 2.1, 2.2). The apertures are quite smooth (Figs. 2.1-2.4). Exine ornamentation is striate perforate (Figs. 2.4, 2.5), consisting of coarse striae with perforations in grooves. Dry (untreated) pollen is prolate with infolded apertures (Fig. 2.6).

Unfortunately there is no pollen data about any *Sibiraea* taxa to compare, but pollen of *Sibiraea* from our research seems to be very similar to those of several other taxa from the Rosaceae family, such as some *Rosa* species (cf. 28), with three-colporate and striate-perforate exine ornamentation. According to these facts, *Sibiraea* fits well into the family Rosaceae, but for more information about palynological connections with typical *Sibiraea altaiensis* further investigation of their pollen should be conducted.

In cases of both researched taxa we can conclude that they have small disjunct populations in the Alpine-Dinaric region, probably differentiated from their relatives long time ago. However, evidence from pollen morphology, as well as from genetic studies (13, 18), indicates a possibility of closer relationships with relatives in the past. Despite this, both taxa developed their own uniqueness, also in palynological sense, and deserved protection in natural habitats as well as conservation, including pollen samples.

Further studies of other endemic plants from Alpine-Dinaric region, as well as investigation of their taxa closely related to *Degenia* and *Sibiraea*, can provide much more information about their taxonomic and phylogenetic relationships and give us more data with regard to their biological conservation and protection.

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