

# Palynological Data of Pollen Grains of *Geranium Sylvaticum*

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**Abstract**—This study is presented for the first time in Albanian literature. For the elaboration processing of pollen grains are used these methods: acetolysis method according Erdtman (1960), acetolysis method according Avetisjan (1950), basic fuchsine method according Smoljaninova & Gollubkova (1953).

The pollen grains of *Geranium Sylvaticum* were characterized by large size. To see the influence of ecological factors and the processing methods of pollen grains, is done the comparison of dimensions of pollen grains of this plant with those of *Geranium mole* and *Geranium asphodeloides* Brun. taken from literature.

From the comparison showed that pollen grains were 3 porate and had the same shape. Pollen grains of *Geranium sylvatica* were greater than those of *Geranium mole* and smaller than those of *Geranium asphodeloides*. Pores were lalongate, wider than long.

Layer of exine was thick and composed by ectexine and endexine. Ektexine was thinner than endexine. Exine's sculpture was reticulate/gemmate.

**Keywords**— Albania, exine, intine, 3 porate.

## I. INTRODUCTION

**P**ALYNOLOGY studies the morphological characteristics of pollen grains as their dimensions (size of equatorial and polar axis, length of furrows and pores etc.); their shape, number of apertures, structure of exine and intine, their thickness, the length of apocolpium, mesocolpium, mesoporum etc. Foreign literature shows that in the size of pollen grains affects many ecological factors and their processing methods (Ducker and Knox, 1985; Surova and Gumbatov, 1986). To see this effect, this study was undertaken.

Numerous studies in the field of palynology have been made by many local and foreign authors. (Basset et al., 1978; Botelli et al., 1982; Gjebrea, 2003; Kallajxhiu, 2011; Kapidani, 1996; Lekli et al., 2008; Pupuleku, 2001; Ricciardelli d'Albore, 1998).

Geraniaceae family includes three genres among which *Geranium* gender. In Albania, it has 25 species. One of them is *Geranium Sylvaticum*. This plant increases in forest, grassland and highland meadows. Flourishing: June to August (Qosja et al., 1992). The plant was taken in Llogara area of Albania, during a field expedition.

Palynological characteristics of *Geranium Sylvaticum* are

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compared to those of *Geranium mole* and *Geranium asphodeloides* taken by literature (Kapidani, 1996; Pupuleku, 2001).

This palynological study is the first Albanian literature.

The aim of this study was:

- The palynological description of pollen grains of *Geranium sylvatica*
- The identification of similarities and differences of pollen grains of this plant against of *Geranium mole* and *Geranium asphodeloides*, taken by literature.

## II. MATERIAL AND METHODS

Pollen grains of *Geranium Sylvaticum* were collected in fresh condition to its habitat of Llogara, in Albania. For identification of the plant, the book "Flora of Albania" was used.

Morphological characteristics of pollen grains were studied by using three analytical methods as follows:

- Acetolysis method according Erdtman (1960),
- Acetolysis method according Avetisjan method (1950),
- Basic fuchsine method according Smoljaninova & Gollubkova (1953).

The first two methods of acetolysis were used to get the best results of the study of sporoderma elements. The method of fuchsine was used to study the shape, size of aperture which in some cases enabled us to identify the sculpture elements of exine.

There were prepared 3-5 microscope slide for each plant by different methods and they were studied by the Digital Microscope/Camera Software, Motic Images Plus 2.0 ML, B1 SERIES. (This microscop save images in JPG, BMP, MIG and TIFF and process the image with a variety of filters). There were presented the microscopic photos of pollen grains of the plant studied in polar and equatorial view with magnification 400 x and 1000 x, taken by KALLAJXHIU Nikoleta. There were presented photographs of respective plant as well.

### A. The Method Of Acetolysis According To Erdtman

The flower or leaf-bud was elaborated in an ethyl alcohol (ethanol) 96° with the aim to separate the other parts of the flower which could be separated inside distilled water. The pollen grains with its granules were dried in a thermostat, and then wetted with an acetolysis mixture (anhydrite acetic and sulfate acid concentration and with pure chemicals in a 9:1 ratio), which was done every time in a repeating way. The test-tubes together with granules and acetolysis mixture were

placed in bathroom at a temperature 70 - 80 °C. The length - time of granules staying in bathroom varied from one kind to another (from 5 to 20 seconds). Then, the test-tubes were centrifuged, whereas the granules were cleaned several times with distilled water. Granules were placed on slide and were observed with a microscope by dropping a drop from glycerin solution and water in a ratio 1:1. Right after the granules were darkened enough, the material was separated in a test-tube by adding 1 - 2 sodium chloride and 1 - 2 concentration sulfuric acid drops till the material became lighter. Then, the second shower with distilled water was done. The material taken through separation and centrifugation was ready to be used as a preparation.

#### B. The Simplified Method Of Acetolysis According To Avetisjan

Granules were placed on slide. Some drops of ethyl alcohol (96%) were dropped on the slide composite. All fat substances of granules created after the alcohol actions were cleaned with blotting - paper. The mixture of acetolysis was prepared every time frequently. The microscope slide composite was treated with 1 - 2 acetolysis solution drops and later on it was warmed up in a thermostat or on the alcoholic lamp flames. The composite was continuously controlled over the warming phase by the microscope, thus it could not get darker then it was allowed. Right after the desired color was reached, a wash - up with ethyl alcohol (70 %) was done. The composite was cleaned up from all residues and fixed with glycerin gelatin which was prepared according to Kissler method, (Erdtman, 1960; Sladkov, 1967).

#### C. The Colored Method Of Basic Fuchsin According To Smoljaninova & Gollubkova

Some alcoholic concentrated drops were added to the granules placed on the microscope slide. In cases of quick evaporation of alcohol some extra drops were added. It was observed that the fat composite of granules were spread from alcohol towards the slide edges. The fat composite was taken away from the slide with blotting paper. After the slide was washed away from residues, the colored solution of basic fuchsin which was prepared according to two variants listed here-below, was added:

- basic fuchsin, alcohol 75 % and phenol in the ratio of 1:700:100
- basic fuchsin, ethyl alcohol 96 % and xylol in the ratio of 1:600:800

Phenol and xylol were used in the transparency growth of markers and were necessary as antiseptic. The color materials were fixed with gelatin glycerin prepared according to Kissler method.

### III. RESULTS

#### Morphological Description Of Pollen Grains

Gjinja: *Geranium L.*

Wood cranesbill – *Geranium Sylvaticum L.*

Hemichryptophyta. Perennials plant. *Geranium Sylvaticum*

flowers have five very similar petals, and are thus radially symmetrical (Qosja et al., 1992).

Pollen grains of *Geranium Sylvaticum* were triporate with prolate - spheroidal contours. In equatorial view, pollen grains had oval frame whereas in polar view they had spheroidal frame. Pores were almost wider than long (alongate). The length of pores varied from 8.16 to 10,2 (8.5)  $\mu$  while the width varied from 12,24 to 16,32 (14,11)  $\mu$ . The layer of exine was bilayer and it was thick. Exine's sculpture was reticulate/gemmate with bacula and gemmae. Bacula formations are fairly developed and sized about 4  $\mu$ . Thickness of exine reaches up to 4  $\mu$ . Ektexine was 1  $\mu$  and endexine was 3  $\mu$  (ektexine was thinner than endexine). Length of pollen grains varied from 58,08 to 65,28 (60,90)  $\mu$  while its width varied from 57,35 to 69,28 (60,5)  $\mu$ .

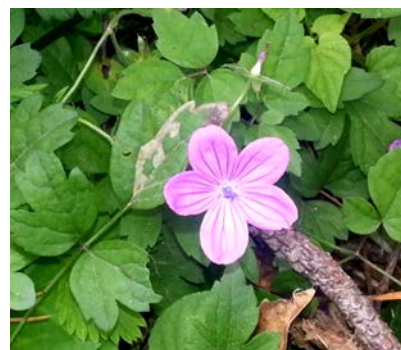


Fig. 1 *Geranium Sylvaticum*

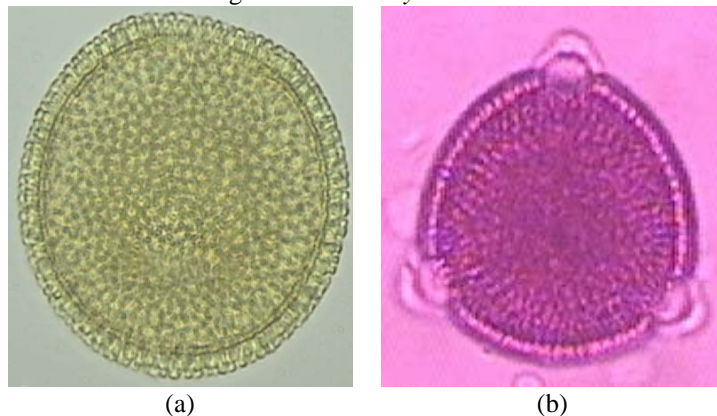


Fig. 2 a. Pollen grains in equatorial view; b. pollen grain in polar view

(Digital Microscope/Camera Software, MO - magnification 1000x (a), 400x (b), Photo: Kallajxhiu, Nikoleta)

### IV. DISCUSSION

By comparing the observed palynological data of the plant of *Geranium sylvatica* with pollen grains of *Geranium mole* and *Geranium asphodeloides* referred to the literature (Pupuleku, 2001; Kapidani, 1996), many similarities and differences between them were identified, as presented in table I.

TABLE I  
DIMENSIONS OF POLLEN GRAINS OF GERANIUM SYLVATICUM, GERANIUM MOLE AND GERANIUM ASPHODELOIDES

The palynological features	Minimum <i>Geranium sylvaticum</i>	Minimum <i>Geranium mole</i>	Minimum <i>Geranium asphodeloides</i>	Maximum <i>Geranium sylvaticum</i>	Maximum <i>Geranium mole</i>	Maximum <i>Geranium asphodeloides</i>
The length of pollen grains	58.08 μ	57.1 μ	75.3 μ	65.28 μ	63.3 μ	95.1 μ
The width of pollen grains	57.35 μ	57.15 μ	75.3 μ	69.28 μ	67.3 μ	95.1 μ
The length of pores	8.16 μ	10.2 μ	20 μ	10.2 μ	20.4 μ	20 μ
The width of pores	12.24 μ	9.2 μ	20 μ	16.32 μ	15.3 μ	20 μ

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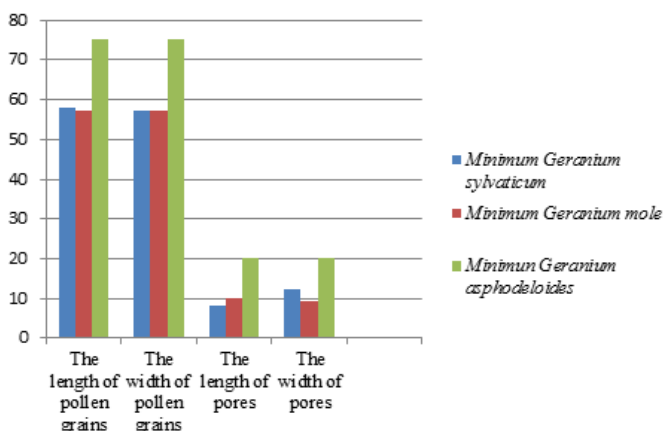


Fig. 3 Chart of minimum dimensions of pollen grains of *Geranium Sylvaticum*, *Geranium mole* and *Geranium asphodeloides*

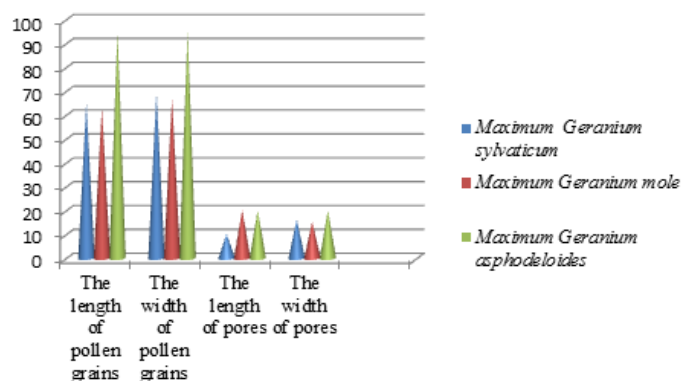


Fig. 4 Chart Of Maximum Dimensions Of Pollen Grains Of *Geranium Sylvaticum*, *Geranium Mole* and *Geranium Asphodeloides*

Based on the palynological features, as indicated in figure 3, minimal dimensions (in length and width) of pollen grains in *Geranium Sylvaticum* were slightly larger than those of *Geranium mole* and much smaller than those of *Geranium asphodeloides*. In *Geranium Sylvaticum*, the length of pores

was smaller than in *Geranium mole* and in *Geranium asphodeloides*. The width of pores was greater than in *Geranium mole* and much smaller than in *Geranium asphodeloides*. In *Geranium Sylvaticum*, pores were lalongate (wider than long). On the contrary, in the Granium mole they were longer than broad. In the *Geranium asphodeloides*, pores were spherical.

TABLE II  
AVERAGE DIMENSIONS OF POLLEN GRAINS OF GERANIUM SYLVATICUM, GERANIUM MOLE AND GERANIUM ASPHODELOIDES

The palynological features	Average <i>Geranium sylvaticum</i>	Average <i>Geranium mole</i>	Average <i>Geranium asphodeloides</i>
The length of pollen grains	60.90 μ	58.9 μ	84 μ
The width of pollen grains	60.5 μ	58.3 μ	84 μ
The length of pores	8.5 μ	16.7 μ	20 μ
The width of pores	14.1 μ	12.1 μ	20 μ
The thickness of exina	4 μ	7 μ	9 μ
The length of bacule	4 μ	3 μ	3 μ
The sculpture of exine	Reticulate gemmate	Reticulate gemmate	Reticulate gemmate

As noted by the data of Table II, the exine structure were presented equivalent in the three species, as reticulate gemmate. Bacule formations were fairly developed. They join in on the regular basis creating polygonal figures, which give the body rippled contours. The length of bacule was greater than that of two other plants.

As indicated in figure 5, the largest dimensions were identified to *Geranium asphodeloides* (in all features – except the length of bacule). Pollen grains of *Geranium Sylvaticum* had length and width greater than those of *Geranium mole* in average dimensions. Thickness of exine was smaller in *Geranium Sylvaticum* and biggest in *Geranium asphodeloides*.

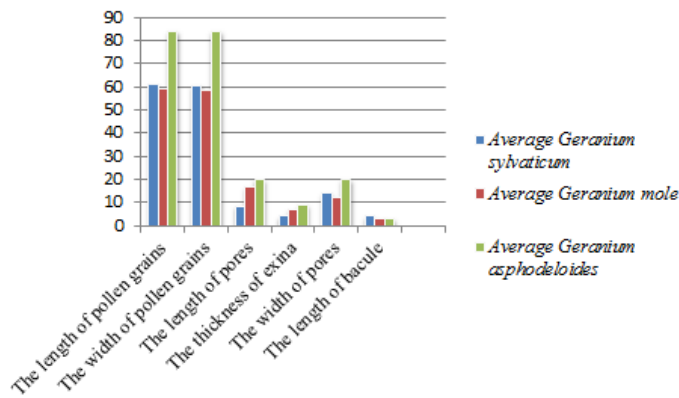


Fig 5. Chart Of Average Dimensions Of Pollen Grains Of *Geranium Sylvaticum* And *Geranium Mole*, *Geranium Asphodeloides*

### V. CONCLUSION

This palynological study of *Geranium Sylvaticum*, concluded as follows:

- Palynomorphological features of *Geranium Sylvaticum* were very similar with those of *Geranium mole* and *Geranium asphodeloides*, in terms of:
  - Pollen grains were triporate (three pores),
  - Pores were wider than longer,
  - The shape of pollen grains was prolate - spheroidal contours,
  - The layer of exine was divided in two underlays: ektexine appeared thinner than endexine.
  - Structure of exine were equal. It was reticulate gemmate.
- There were differences between them only in terms of the dimensions of pollen grains. They were as follows:
  - Exine thickness of *Geranium Sylvaticum* were smaller than that of *Geranium mole* and *Geranium asphodeloides*,
  - The length and width of pollen grains of *Geranium Sylvaticum* were greater than those of *Geranium mole* and smaller than those of *Geranium asphodeloides*.
  - The length of pores of *Geranium Sylvaticum* was less than that of *Geranium mole* and *Geranium asphodeloides*.

### REFERENCES

[1] Avetisjan, B. M., (1950): Uproshennij acetolinij metod obrabotniki pilci. Botanicheskii Zhurnal. 35(4): 385-386.  
 [2] Basset, I.J., C.W., Crompton, J.A. Paemelee (1978): An atlas of airborne pollen grains and common fungus spores of Canada. Canada Dept. of Agriculture. Monograf Nr.8.  
 [3] Bottelli, R., Falagiani, P., Galimberti, M., Lenzi, G., Pacini, E., Rolo, J. (1982): I pollini e la Pollinosi, PADOVA, 10-13, 15-20.  
 [4] Ducker, C.S., Knox, B.R. (1985): Pollen and people. Biotechnology and ecology of Pollen, New York, 399-404.  
 [5] Erdtman, G. (1956): Morfologija pilci i sistematika rastenij, Angiosperma. Moskva, 12-14, 25-85.

[7] Gjebrea, E. (2003): Identifikimi dhe përqëndrimi në ajër i poleneve kryesore alergjizues në Tiranë dhe kalendari polenik.  
 [8] Kallajxhiu, N. (2011): Studimi alergopalinologjik i bimëve alergjike të rrethit të Elbasanit dhe i alergjive të shkaktuara prej tyre. Rama Graf. Elbasan.  
 [9] Kapidani, G. (1996): Bazat e palinologjisë. Spore dhe polene të disa bimëve të sotme të Shqipërisë. Seiko, Elbasan.  
 [10] Pupuleku, B. (2001): Studimi melissopalinologjik i mjaltërave të rajonit Elbasan dhe i poleneve të disa bimëve mjaltore. Disertacion.  
 [11] Qosja, Xh., Papparisto, K., Vangjeli, J., Demiri, M., Balza, E., Marika, A. (1992): Flora of Albania. Volume II, Tirana, Albania, 201-205.  
 [12] Ricciardelli d'Albore, G., (1998): Mediterranean melissopalynology. Universita degli studi di Perugia, Istituto di Entomologia agraria, Facolta di Agraria, Perugia.  
 [13] Sllatkov, A.N. 1967. V vedenie V sporovo pilcevo analiz.  
 [14] Smoljaninova, L. A., & Gollubkova, V. F. (1953): L. A. K. Metodike issledovani pilci. Doklady Akademia Nauk SSSR T LXXXVIII. 1:125-126.  
 [15] Surova, T.D., Gumbatov, Z.J. (1986): Ultrastruktura obovlocka pilcevi zeren Kavkazni  
 [16] prectavitel Taxus baccata (Taxaceae), Bot. Zhurnal T. 71, Nr.7, 886-888.

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