



**PALYNOLOGICAL STUDY OF POLLEN GRAINS OF ALBANIA'S ENDEMIC PLANT *Festucopsis serpentini* (C.E. HUBBARD) MELDERIS AND *Lilium martagon* L. IN THREE DIFFERENT HABITATS**

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**SYNOPSIS**

**Key words:**

Pollen grains,  
exine,  
intine,  
monade,  
caryology,  
*Festucopsis*,  
*Lilium*,  
Albania.

This study provides data of pollen grains of Albania's endemic plant of *Festucopsis serpentini* (C. E. Hubbard) Melderis, collected in fresh condition to their habitat of Librazhd, and the plant of *Lilium martagon* L. collected in three different habitats: Guri i Zi; Ostrovicë and Mali me Gropa.

Palynological data for the plants of *Festucopsis serpentini* and *Lilium martagon* were provided for the first time in the Albania's palynological literature. The pollen grains of these plants were determined through the identification of a large number of valid elements, and a diligent study of various palynomorphological indicators. Referring to the palynomorphological study of the plant *Lilium martagon* L., it was concluded that the size of pollen grains varied from one habitat to another, influenced by ecological factor.

Caryological data of the Albanian endemic plant *Festucopsis serpentini* (C.E. Hubbard) Melderis, whose chromosome number was  $2n = 14$ , and the plant of *Lilium martagon* L.,  $2n = 24$  (Turku, 2007), were provided for the first time in this study as well.

**INTRODUCTION**

The morphological characteristic features of the pollen grains of covered seed (angiospermae) plants were as follows: position and construction of apertures,

sporoderm feature, polarity and symmetry, shape and dimensions (Kapidani, 2005; Pupuleku, 2002).

Pollen grains of the Gramineae family were mainly single pores. Referring to the Albania's Flora (Vangjeli et al., 2000) Gramineae family in Albania includes 93 specimens.

*Festucopsis serpentini* is an Albanian endemic specimen located in serpentine rocks, mainly in South - East Albania. Its pollen grains samples were collected in fresh condition, in Librazhd area, on 06. 06. 2007.

Pollen grains of the Liliaceae family were mainly single furrow. Referring to the Albania's Flora (Vangjeli et al., 2000), there are 25 specimens included to the Liliaceae family in Albania. The Genus *Lilium*, in Albania involves 4 species as follows: *albanicum*, *candidum*, *chalcedonicum* and *martagon*.

The scope of this study was the palynomorphological description of pollen grains of Albania's endemic plant as follows: *Festucopsis serpentini* (C. E. Hubbard), Melderis; and *Lilium martagon* L.

## MATERIAL AND METHODS

Pollen grains of the plant of *Lilium martagon* were collected in fresh conditions to three different habitats as follows: Guri i Zi; Ostrovicë; and Mali me Gropa, and those of the plant of *Festucopsis serpentini* to the Librazhd area. Morphological characteristics of pollen grains were studied by using three analytical methods as follows:

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

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

### 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.

#### 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 than 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 Kisser method (Erdtman, 1960; Sladkov, 1967).

#### THE COLORED METHOD OF BASIC FUCHSINE 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 fuchsine which was prepared according to two variants listed here-below, was added:

- 1) basic fuchsine, alcohol 75 % and phenol in the ratio of 1:700:100
- 2) basic fuchsine, 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 Kisser method.

#### FIXTURE OF PREPARED COMPOSITES

The fixture of prepared composites was realised by using the method of glue-preparations through gel-glycerine. The gel-glycerine was prepared based on the

Kisser method by using 50 gr of gel, 175 ml of distilate water, 150 gr glycerine and 7 gr phenol. Once the distilate water was heated up to 50°C, the gel was dropped into it. It was mixed up several times till melted properly. Then the glycerine and the composite were added and boiled till the liquid became thicker and viscose. After the phenol was added to the mixture, a uniform melted composition was taken. The prevention of air bubbles that might emerge during the process of composite preparation was made by warming up in advance all equipment used over the process. The final composite was isolated to the edges of microscope slide with spray or paraffin and after 3 days it was ready to be used and stored.

In order to identify pollen grains, the characteristics of openness, character, and shape should be taken into consideration.

There were prepared 3 - 5 microscope slide for each plant by different methods and they were studied by the microscope "Biollamp". There were presented 4 microscopic photos of pollen grains of the plants studied in their respective habitats with magnification x 350, taken by PUPULEKU Blerina. There were presented 4 photos of respective plants as well, taken by camera Sony 12.1, TURKU Silvana.

## RESULTS AND DISCUSSION

### MORPHOLOGICAL DESCRIPTION OF POLLEN GRAINS

Family: Gramineae

Genus: *Festucopsis* (C. E. Hubbard) Melderis

***Festucopsis serpentini*** (C. E. Hubbard) Melderis (*Brachypodium serpentini* C. E. Hubbard) Figs. 1 and 2.

Hemichryptophyta. Perennial grass plants, in batches, up to 40 cm high. Found in soil and serpentinerocks, mainly on South East Albanian. Flowering: May - July (Vangjeli et al., 2000).  $2n = 14$ . Albania's endemic plant. The fresh pollen grains were taken in Librazhd area, on 06.06.2007.

Pollen grains were monades with single pore. The pollen grains shape varied from oblate to spheroid ( $P/E = 1.01$ ). In the polar position pollen grains had almost circular frame, whereas in the equatorial position they had oval frame.

Pores were circular and with contour. Pore diameter varied from 4.5 - 6.3 (5.4) $\mu$ . By processing the basic fuchsine, at the center of pore could be identified a small piece like "island" that was part of exine.

The exine was doubled layer with a fine granulose sculpture. In the area of pores the exine thickened slightly. The size of the exine layers was almost equal. Citoplazma was very smooth. The thickness of exine varied from 1.0 - 1.6 $\mu$ .

Diameter of pollen grains varied from 29.2 - 33.1 (31.2) $\mu$ .



Figure 1: *Festucopsis serpentini* – Librazhd.  
(Sony 12.1, Photo: TURKU S.)



Figure 2: Single pore pollen grains.  
(Biollamp microscope x 350, Photo:  
PUPULEKU B.)

Family: Liliaceae

Genus: *Lilium* L.

***Lilium martagon* L.**

Geofite bulboze. Bulboze perennial grass plant. In beech forests particularly, mountain meadows, shrubby. Flowering: June - July (Vangjeli, et al., 2000).  $2n = 24$ . Eurasian. Fagetalia. Honey plant and colorant. Figs. 3 and 4.

The fresh pollen grains were taken in Guri i Zi, on 27. 06. 2009, in Ostrovicë, on 10. 07. 2009, and in Mali me Gropa, on 18. 07. 2009.

Pollen grains were monades with single furrow. In the polar and the equatorial position pollen grains had oval couture. The furrow was smooth and went up to the pole. The exine of pollen grains was mono layer. Its sculpture looked like a group of pin-heads, whose heads form opened reticulum. Cell reticulum size varied.

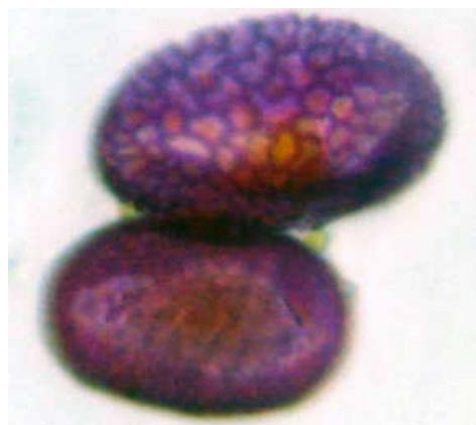
In Guri Zi habitat: The wideness of furrow varied from 10.2 to 8.0 (14.1) $\mu$ . The thickness band of exine was 1.9 - 2.5 (2.2) $\mu$ . The height of pollen grains varied from 65.2 to 83.6 (71.4) $\mu$ . The wideness of pollen grains varied from 41.8 to 56.1 (47.1) $\mu$ .

In Ostrovicë habitat: The wideness of furrow varied from 8.6 to 15.3 (11.1) $\mu$ . The thickness band of exine was 1.4 - 2.3 (1.8) $\mu$ . The height of pollen grains varied from 60.2 to 77.5 (67.9) $\mu$ . The wideness of pollen grains varied from 37.8 to 53.1 (44.3) $\mu$  (Figures 5, 6).

In Mali me Gropa habitat: The wideness of furrow varied from 8.6 to 15.9 (12.6) $\mu$ . The thickness band of exine was 1.8 - 2.5 (2.3) $\mu$ . The height of pollen grains varied from 59.2 to 85.7 (72.4) $\mu$ . The wideness of pollen grains varied from 39.8 to 60.2 (47.8) $\mu$  (Figures 7, 8).



**Figure 3: *Lilium martagon* - Guri i Zi.**  
(Sony 12.1, Photo: Turku, S.)



**Figure 4: Single furrow pollen grains.**  
(Biollamp microscope x 350,  
Photo: Pupuleku, B.)



**Figure 5: *Lilium martagon* – Ostrovicë.**  
(Sony 12.1, Photo: Turku, S.)



**Figure 6: Single furrow pollen grains.**  
(Biollamp microscope x 350,  
Photo: Pupuleku, B.)



**Figure 7: *Lilium martagon* - Mali me Gropa.**  
(Sony 12.1, Photo: Turku S.)



**Figure 8: Single furrow pollen grains.**  
(Biollamp microscope x 350, Photo: Pupuleku B.)

## RESULTS

By comparing the observed palynological data (size of pollen grains, furrow and thickness of exine) of the plant of *Lilium martagon* L. at three different sites such as Guri i Zi, Ostrovicë and Mali me Gropa, many similarities and differences between them were identified, as presented in table 1.

**Table 1: Dimensions of pollen grains of *Lilium martagon* in three habitats.**

The palynological feature	Min. Guri i Zi	Min. Ostrovicë	Min. Mali me Gropa	Max. Guri i Zi	Max. Ostrovicë	Max. Mali me Gropa	Av. Guri i Zi	Av. Ostrovicë	Av. Mali me Gropa
The height of pollen grains	65.3	60.2	59.2	83.6	77.5	85.7	71.4	67.9	72.4
The wideness of pollen grains	41.8	37.7	39.8	56.1	53	60.2	47.1	44.3	47.8
The wideness of furrow	10.2	8.6	8.6	18	15.3	15.9	14	11.1	12.6
The thickness of exine	1.8	1.4	1.8	2.5	2.2	2.5	2.2	1.8	2.3

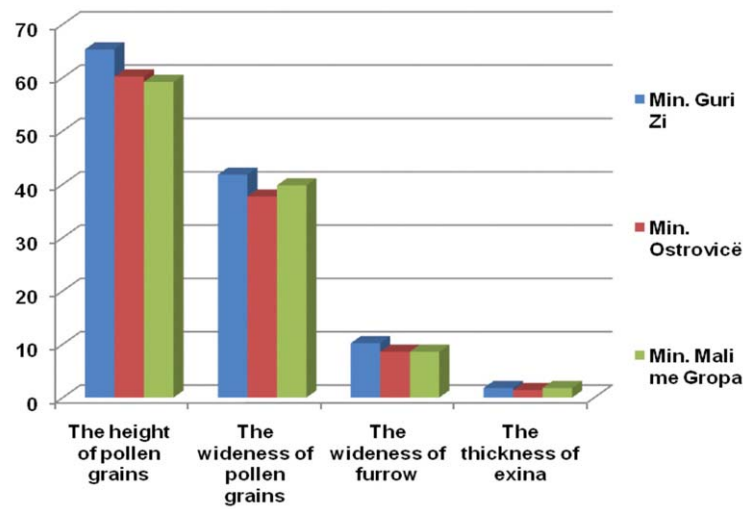


Figure 9: Minimum dimensions of pollen grains of Lilium martagon in three habitats.

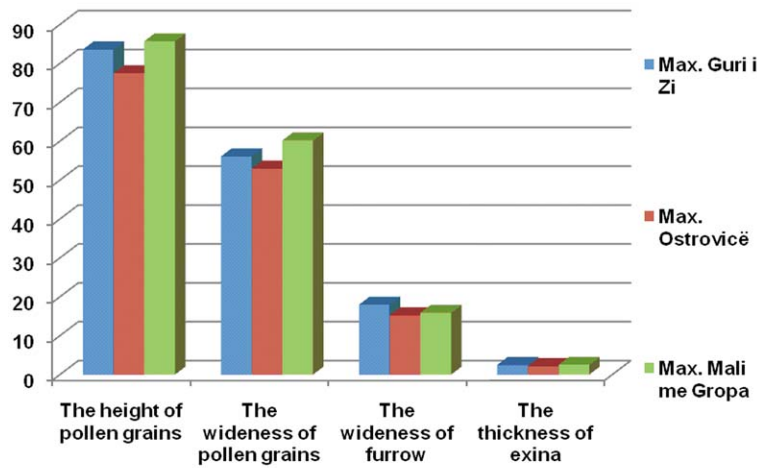


Figure 10: Maximum dimensions of pollen grains of Lilium martagon in three habitats.

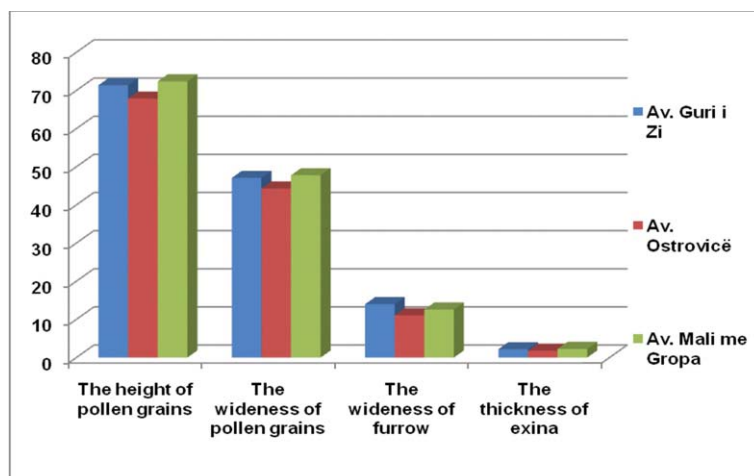


Figure 11: Average dimensions of pollen grains of Lilium martagon in three habitats.

As seen from the chart 1, the pollen grains from the Mali me Gropa were lower in the length of pollen grains and the furrow, whereas in the Ostrovicë area the pollen grains were smaller in the width of pollen grains, furrow and thickness of exine.

The same thing was found on the average size of the pollen grains morphological indicators for the pollen grains of Mali me Gropa area, which appeared to be larger than those of the other two sites, except that the furrow width was greater in Guri i Zi area than the other two. In terms of maximum size, it was found out that the pollen grains of Mali me Gropa area were larger than other two sites, equal to the thickness of the exine for the two habitats: Mali me Gropa and Guri i Zi and the highness of furrow in Guri i Zi area was the highest.

It is very well known the impact of ecological factor to the pollen grains dimensions and distortion (Ducker & Knox, 1985; Surova & Gumbatov, 1986).

Based on the palynological characteristics, the pollen grains of the plants studied can be classified in the forms as follows:

One pore pollen grains - *Festucopsis serpentini*;

One furrow pollen grains - *Lilium martagon*.

Based on the caryological data of Albania's endemic plant *Festucopsis serpentini* (C. E. Hubbard) Melderis, the chromosome number resulted  $2n = 14$  and for the plant *Lilium martagon* L., the chromosome number is  $2n = 24$  (Turku, 2007).

## CONCLUSIONS

Pollen grains of *Festucopsis serpentini* plants were single pore and pollen grains of *Lilium martagon* plants were single furrow.

Palynomorphological features of the pollen grains for the plant *Lilium martagon* collected at sites of Guri i Zi, Ostrovicë and Mali me Gropa were very similar among them.

From the size feature the pollen grains of the plant *Lilium martagon* collected to the above mentioned sites, were different because of the influence of ecological factors. The size of the pollen grains of the plant *Lilium martagon* collected to Mali me Gropa area for three palynomorphological features (length, width of the pollen grains and exine thickness) was larger than those of other two sites, whereas the width of pollen grains furrow collected to the Guri i Zi area was larger than the other two areas. Referring to two palynomorphological features (pollen grains length and width of the furrow) the size of pollen grains collected to the Mali me Gropa was smaller than those of other sites. Considering three palynomorphological features

(width of pollen grains, furrow and thickness of exine), the size of pollen grains collected to Ostrovicë area was smaller than those of other areas.

The chromosome number of Albanian endemic plant *Festucopsis serpentini* (C. E. Hubbard) Melderis was  $2n = 14$  and for *Lilium martagon* L. the chromosome number was  $2n = 24$ .

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