

Palynological Comparison of Pollen Grains of *Ranunculus psilostachys* With Those of *Ranunculus Bulbosum* And *Ranunculus Sardous*

¹Nikoleta Kallajxhiu, ²Gëzim Kapidani, ¹Peçi Naqellari, ¹Blerina Pupuleku,
¹Silvana Turku and ¹Ermelinda Gjeta

¹Department of Biology, FSHN, “A. Xhuvani” University of Elbasan, Albania

²Department of Biology, FSHN, University of Tirana, Albania

ABSTRACT: In this study is presented palynomorphological description of pollen grains of *Ranunculus psilostachys* Griseb., Ranunculaceae family. Fresh material of this plant is taken in Llogara Mount, in Albania, in June 2014. Important morphological features as symmetry, form, size, aperture (type, number), surface pattern, sexine and exine thickness were examined and described using Microscope/Camera Software, MotiC Images Plus 2.0 ML, B₁ Series. To see the influence of ecological factors and laboratory processing method used, is done the comparison of palynomorphological features of pollen grains of *Ranunculus psilostachys* with those of *Ranunculus bulbosum* and *Ranunculus sardous*, taken by the literature. By comparison made between them, showed that pollen grains had similarities in terms of the number of furrows (tricolpate). Pollen grains of *Ranunculus psilostachys* were smaller in length and width than those of *Ranunculus bulbosum* and *Ranunculus sardous*. Furrows were shorter and wider than those of pollen grains in *Ranunculus bulbosum*. Mesocolpium of *Ranunculus psilostachys* is more extensive than the two other types. The layer of exine in *Ranunculus psilostachys* was thicker than that of *Ranunculus bulbosum* and *Ranunculus sardous*. The sculpture of exine was granulate, as in *Ranunculus sardous*, whereas in *Ranunculus bulbosum* was verrucate sculpture with small verrucae.

KEYWORDS: 3 colpate, exine, spheroidale, Llogara, Albania

I. INTRODUCTION

In the flora of Albania, Ranunculaceae family includes 15 genres. One of these is the *Ranunculus L.* which has about 40 plant species. They are one or perennial plants (Paparisto et al., 1988). Pollen grains of some genus *Ranunculus* could be a cause of allergic diseases in susceptible persons by allergic factor (Moore & Webb, 1978; Mandrioli & Puppi, 1978; Ciampolini & Cresti, 1981; Lewis & Lewis, 1977). There are many palynological studies about this family representatives from local and foreign authors (Ciampolini & Cresti, 1981; Kapidani, G., 1998; Pupuleku, B., 2002; Zekaj et al., 2002; Turku, S., 2007; Kallajxhiu, N., 2011). The pollen grains of *Ranunculus psilostachys*, found in the area of Llogara is studied for the first time in our country. Llogara is located about 40 km southeast of the city of Vlora, the spatial boundary between the Adriatic and Ionian sea. It is declared a national park by the Albanian government in 1966. This park covers about 1,010 ha. Height ascending increase from 470 m to 2018 m. There is a very rich herbaceous vegetation, bushes and woods where stands the monument of nature “pine flag”. From literature is showed that ecological factors such as height above sea level, temperature and pressure of ambient impact on the size of pollen grains (the length of the equatorial and polar axis), of furrows and pores, mesocolpium, apocolpium etc. but did not affect in their external form and in the number of openings of exine. In the size of pollen grains affect and the processing methods of pollen grains in the laboratory (Ducker & Knox, 1985; Surova & Gumbatov, 1986). To see exactly this effect, I have conducted this study.

II. MATERIAL AND METHODS

Pollen grains of *Ranunculus psilostachys* were collected in fresh conditions to its habitat of Llogara, in Albania during field expeditions conducted with students, on 19th of June 2014. 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 fuchsin method according 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 fuchsin was used to study the form, size of aperture, which in some cases enabled us to identify the sculpture elements of exine. The average sizes of the pollen grains were determined by measuring 31 pollen grains. There were prepared 3-5 microscope slide for the pollen grains by different methods and they were studied by the Digital Microscope/Camera Software, Motic Images Plus 2.0 ML, B1 Series. (This microscope saves 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 400x and 1000x, taken by KALLAJXHIU Nikolaeta.

III. THE METHOD OF ACETOLYSIS ACCORDING TO ERDTMAN

The flower or leaf-bud was elaborated in ethanol 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, 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 ethanol were dropped on the slide composite. All fat substances of granules, created after the ethanol 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 Kisser method.

The Colored Method of Basic Fuchsin According to Smoljaninova & Gollubkova: Some ethanol concentrated drops were added to the granules placed on the microscope slide. In cases of quick evaporation of ethanol, 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:

- A. Basic Fuchsin, Alcohol 75 % and Phenol in the Ratio of 1:700:100
- B. 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 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 distillate water, 150 gr glycerine and 7 gr phenol. Once the distillate 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.

IV. RESULTS

The study area belongs palynology. Below is showing the morphological description of pollen grains of *Ranunculus psilostachys*.

Family: **Ranunculaceae**

Gender: **Ranunculus L.**

***Ranunculus psilostachys* Griseb.**

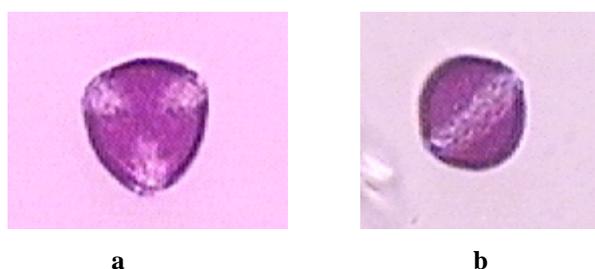
It is a hemychryptophyta and perennial herbaceous plant; simple or slightly branched, with root tuber. Leaves were with hairs mainly silk underneath, longer than broad, cut threesome. Receptacle was oblong, more or less cylindrical at the time of the fruitification. This plant grows in grassy places, mainly in the mountainous areas and blooms in the months from June to July. It is a sub-balkanical plant (Paparisto *et al.*, 1998).

Pollen grains of this plant were tricolpate. The shape of pollen grains according to the contours was prolate spheroidal ($P = 1.02$). In equatorial view, pollen grains had prolate frame whereas in polar view they had circular frame. The length of pollen grains varies from 24.6 to 28.29 (25.59) μ . The pollen grains width ranges from 24.19 to 26.24 (25.01) μ . The furrows were long, with granulate membrane and they were long enough to go to the pole. Their width reaches up to 8.26 μ while the length goes up to 11.27 μ .

Mesocolpium amounts to 17.30 μ . The exine had doubled thin layers with granulitic sculpture. Its thickness reaches up to 2.29 μ . Ektexine is slightly thicker than endexine.



Figure 1: *Ranunculus psilostachys*



a

b

Figure 2: Pollen grains in a) polar view; b) equatorial view prepared with the colored method of basic fuchsin according to Smoljaninova & Gollubkova (Digital Microscope/Camera Software, MO-magnification 400x, Photo: Kallajxhiu, Nikoleta)



c

d

e



Figure 3: Pollen grains c, d, e in polar view; f, g, k in equatorial view prepared with the method of acetolysis according to Erdtman (Digital Microscope/Camera Software, MO-magnification 1000x,

Photo: Kallajxhiu, Nikoleta)

V. DISCUSSION

By comparison of the results obtained about the pollen grains of *Ranunculus psilostachys* to those of *Ranunculus bulbosum* and *Ranunculus sardous* taken by literature (Kallajxhiu, N., 2011; Zekaj *et al.*, 2002; Turku, S., 2007) were identified very similarities and differences between them. There are shown in the tables 1, 2 and 3, here below.

Table I: Dimensions of pollen grains of *Ranunculus psilostachys* compared with those of *Ranunculus bulbosum*

The palynological features	Average Dimension of <i>Ranunculus psilostachys</i>	Average Dimension of <i>Ranunculus bulbosum</i>
The length of pollen grains	25,59 μ	32,25 μ
The width of pollen grains	25,01 μ	30,86 μ
The length of furrow	11,27 μ	31,86 μ
The width of furrow	8,26 μ	4,47 μ
Mesocolpium	17,30 μ	21,90 μ

Table II: Dimensions of pollen grains of *Ranunculus psilostachys* compared with those of *Ranunculus sardous*

The palynological features	Average Dimension of <i>Ranunculus psilostachys</i>	Average Dimension of <i>Ranunculus sardous</i>
The length of pollen grains	25,59 μ	28 μ
The width of pollen grains	25,01 μ	27,6 μ
Mesocolpium	17,30 μ	21,4 μ

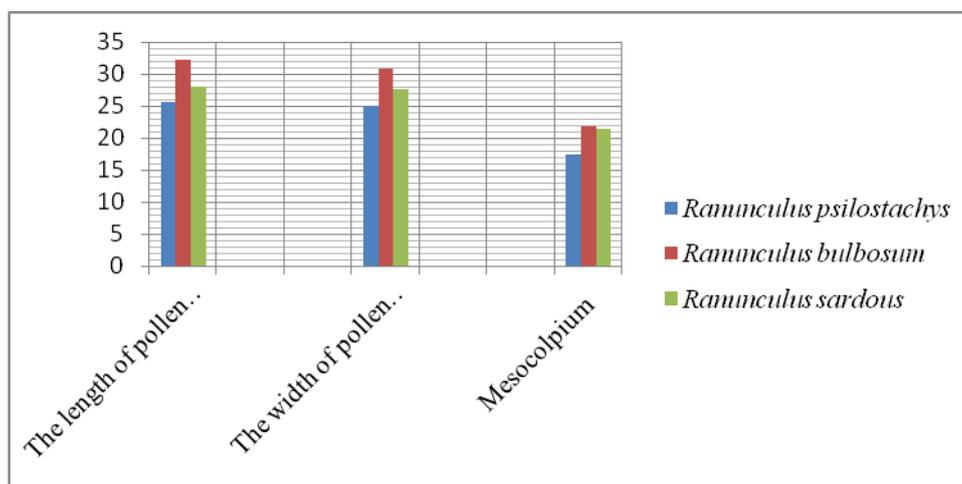


Figure 4: Chart of average dimensions of pollen grains of *Ranunculus psilostachys*, compared with those of *Ranunculus bulbosum* and *Ranunculus sardous*

Based on the palynological features, as indicated in the Fig. 4, it was noted that the pollen grains of *Ranunculus psilostachys* were smaller than those of two other plants. Furrows of pollen grains in *Ranunculus psilostachys* were shorter but wider than those of two other types of pollen grains (indicated in the figure 5). Also, mesocolpium (the distance between two furrows) is greater than that of *Ranunculus bulbosum* and *Ranunculus sardous*.

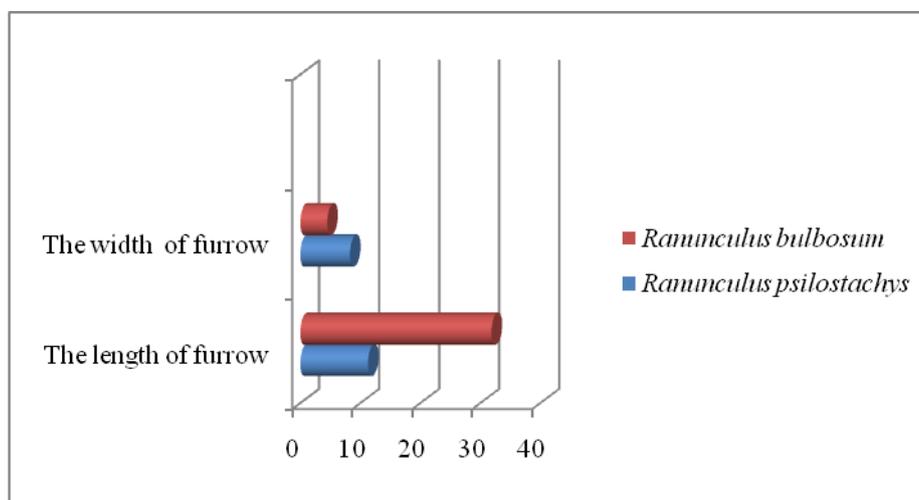


Figure 5: Chart of average dimensions of furrows in *Ranunculus psilostachys*, compared with those of *Ranunculus bulbosum*

Table III: Dimensions of thickness of exine compared of those of two other plants and the sculpture of exine

The palynological features	Average Dimension of <i>Ranunculus psilostachys</i>	Average Dimension of <i>Ranunculus sardous</i>	Average Dimension of <i>Ranunculus bulbosum</i>
The thickness of exine	2.29 μ	2.1 μ	2.1 μ
The sculpture of exine	Granulate	Granulate	Verrucate

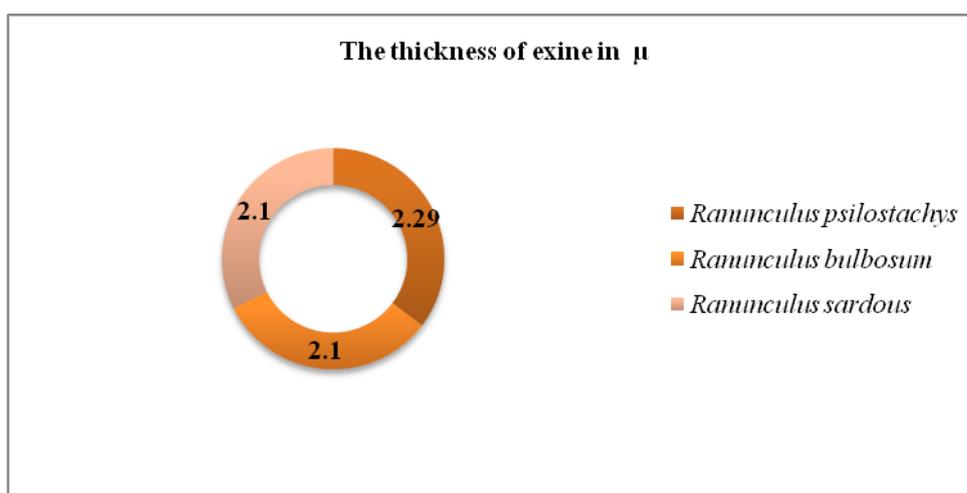


Figure 5: Chart of dimensions of exine

As indicated in the table 3, the exine of pollen grains of *Ranunculus psilostachys* was the same as the sculpture of exine of *Ranunculus bulbosum* but it differed from exine of *Ranunculus sardous* which that appear associated with small verrucae. The layer of exine of *Ranunculus psilostachys* was slightly thicker than the two others.

VI. CONCLUSIONS

The palynological study of *Ranunculus psilostachys* showed that there were changes and many similar in palynomorphological features with 2 other species. Pollen grains of *Ranunculus psilostachys* were smaller in size than those of two other plants except of furrows that were shorter but wider.

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