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Pollen morphology of *Hyacinthella leucophaea* ssp. *atchleyi* (ASPARAGACEAE) and *Valeriana* sp. (VALERIANACEAE) of Shebenik National Park, Albania

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Abstract

In this study, the palynological description of three species taken in one of the most important scientific, economic, historical and cultural parks of Albania, Shebenik National Park, is presented for the first time in the albanian literature.

The studied plants are: Hyacinthella leucophaea ssp. atchleyi (A. K. Jacks. & Turrill) K. Perss & Jim Perss., Valeriana officinalis L. and Valeriana tuberosa L. For each of them, the main palynological features including here the shape of the pollen grains, their dimensions, the length of the equatorial and polar axis, the type and number of apertures of exine, its sculpture and structure, have been determined.

The results showed that the pollen grains of Hyacinthella leucophaea ssp. atchleyi were monocolpate. On the other hand, the pollen grains of Valerina officinalis and Valeriana tuberosa were tricolpate.

The ornamentation type of exine was reticulate in pollen granis of Hyacinthella leucophaea ssp. atchleyi, echinate - microechinate with verrucae patterns in Valeriana tuberosa and echinate in Valeriana officinalis.

Keywords: *pollen morphology, tricolpate, monocolpate, reticulate, echinate, Shebenik National Park*

INTRODUCTION

Hyacinthella leucophaea is a European species widely distributed in Greece, Bulgaria, Romania and Serbia (Diklic, I., 1975; Strid, A., 1986; Assyov & Petrova, 2006). It was found for the first time in Albania in 2007 (Barina *et al*., 2010).

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For the genus Hyacinthella, Albanian flora comprises only two species, *H. leucophaea* and *H. dalmatica* (Baker) Chouard (Gjeta *et al*., 2022). There are many palynological studies by foreign authors for representatives of the Asparagaceae family and especially of the genus *Hyacinthella* (Pehlivan, S. & Özler, H., 2003; Karabacak, O., Erez, M.E., Pınar, S.M. & Fidan, M., 2012; Tekin, M. & Meriç, Ç., 2013; Ferrauto, G. & Pavone, P., 2016; Coşkuner, M., 2019; Heidarian, M., Masoumi, S.M. & Amiri, S., 2021; Aydan Acar Şahin & Hüseyin Eroğlu, 2022). These studies have shown that the pollen grains of the *Hyacinthella* genus are monocolpate and their shape varies from suboblate to oblate.
The other two studied plants belong to the Valerianaceae family. Based on the flora of Albania, the Valerianaceae family includes 3 genera: *Valerianella* Miller, *Valeriana* L. and *Centranthus* DC. The genus V*aleriana* has about 7 species: *Valeriana officinalis*, *Valeriana dioscoridis*, *Valeriana tuberosa*, *Valeriana montana*, *Valeriana bertiscea*, *Valeriana crinii* and *Valeriana saxatilis* (Qosja *et al*., 1996). Based on the palynological studies conducted by many foreign authors, it was concluded that the pollen grains of the genus *Valeriana* were tricolpate (Clarke, G.C.S. & Jones, M.R., 1977; Diez, M.J., 1984; Tsarenko, O.M., Tsymbalyuk, Z.M., Bulakh, O.V., Nitsenko, L.M., 2020).

MATERIAL AND METHODS

The study material was obtained during the field trips compiled with the specialists in the area of botany from different plant individuals, in the Shebenik National Park area in Albania. Their determination was made by the botanist Ermelinda Gjeta.

This park is located in Librazhd District in the Elbasan Region, on the border with Northern Macedonia and has a total area of 34 507.9 ha, where with Decision of Council of Ministers (DCM) no. 59 dated 26.01.2022 to the previous surface of 33 927.66 ha was added 589 ha of new surface.

The Park ranges in altitude from 300 to 2,200 m above sea level and includes a diversity of climatic conditions, geological types, landscapes, habitats, plant and animal species. "Shebenik" National Park represents an important scientific, economic, historical and cultural role.

The flowers and their buds were carefully taken from individuals of different populations. To carry out the palynological study, several pollen preparations have been prepared for each of them.

Two methods have been used for the preparation of pollen slides: acetolysis according to Erdtman (1960) and basic fuchsine method according to Smoljaninova & Gollubkova (1953). The Kisser method was used to fix the slides (Kisser, 1935).

For each species, the measurements of 31 pollen grains were made and the dimensions of the main palynological features such as the length and width of the polar and equatorial axis, the length and width of colpus, mesocolpium, apocolpium, the thickness of the exine and intine layer were measured. The structure of exine of the pollen grain has been determined too.

For conducting the study, a Biological Microscope (Motic BA310 Series LED - Digital) was used. The study was accompanied with photos of species in their habitat and microscopic images of pollen grains in the polar and equatorial view.

This study is a novelty in the Albanian literature as these species have been studied for the first time from the palynomorphological aspect in Albania.

The aim of this study is to identify the palynomorphological features of the pollen grains of the species *Hyacinthella leucophaea* ssp. *atchleyi* (A. K. Jacks. &

Turrill) K. Perss & Jim Perss.,*Valeriana tuberosa* and *Valeriana officinalis* as well as to notice the differences and similarities between those and other species of the same genus studied by other authors.

RESULTS AND DISCUSSION

Morphopalynological description
Family: **Asparagaceae Juss.**
Genus: ***Hyacinthella* Schur.**
Subspecies: *Hyacinthella leucophaea* ssp. *atchleyi* (A. K. Jacks. & Turrill) K. Perss & Jim Perss

Subspecies *Hyacinthella leucophaea* ssp. *atchleyi* is a herbaceous glabrous, bulbous, perennial plant, flowering period March – April. It is is a new taxon for albanian flora, and a new location to the species as well, found in Ruen located at 41° 8'33.41"N and 20°35'18.96"E (Gjeta *et al*., 2022).

The pollen grains are monocolpate. In the equatorial view, they have an elliptical shape. The length of the equatorial axis varies from (16 - 29) 22.8 µm while that of the polar axis varies from (30 - 40) 33.6 µm. The contour shape of the pollen grain is longitudinal (prolate) (P/E = 1.47).

The colpus is long and it reaches the pole. Its width varies from (8 - 19) 12.6 µm. The exine layer reaches up to 1.5 µm. Exine sculpture is reticulate.

Fig. 1. *Hyacinthella leucophaea* ssp. *atchleyi* (A. K. Jacks. & Turrill) K. Perss & Jim Perss (in its habitat)

a b

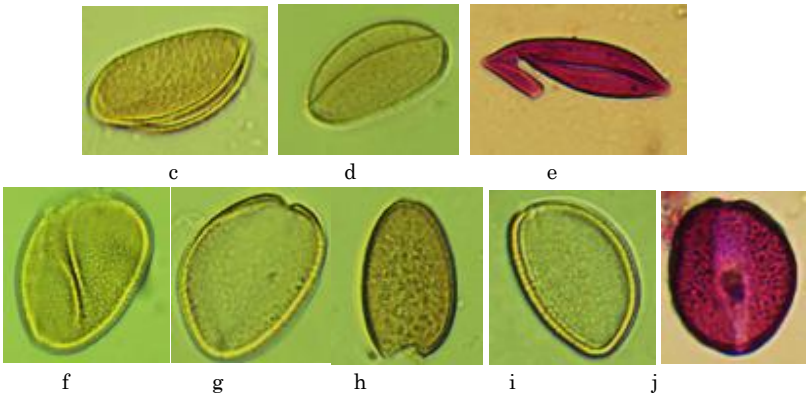

Fig. 2. *Hyacinthella leucophaea* ssp. *atchleyi* pollen grains a. exine ornamentation (reticulate) - basic fuchsine method; b. pollen grains in lateral view x400 – acetolysis method; c, d. pollen grains in lateral view x400 – acetolysis method; e. pollen grain in equatorial view with sulcus x400 - basic fuchsine method; f, g, h, i. lateral view x400; i. equatorial view x400– acetolysis method;j. colpus- basic fuchsine method. (photo: **Kallajxhiu, N**.)

Family: **Valerianaceae**
Genus: ***Valeriana* L.**
Species: *Valeriana tuberosa* L.

Valeriana tuberosa is a perennial hemicryptophyte plant with short rhizomes and tubers. Its flowers are hermaphroditic and pink in color. The plant grows in dry grassy places, on the rocks of mountainous areas and blooms in May - August (Qosja *et al*., 1996). The material was taken in Ruen, Shebenik National Park, Albania. Ruen area is located in the south-eastern part of this national park, next to the border with the North Macedonia.

The study shows that the pollen grains of *Valeriana tuberosa* are tricolpate monads. The length of the polar axis varies from 40 - 61 (45.2) µm while the length of the equatorial axis varies from 40 - 59 (45.5) µm. Based on the dimensions determined by the study as well as in the literature, it results that the pollen grains of *V. tuberosa* are medium or large-sized (25 - 50; 50 - 100) µm (Kapidani, G., 1996).

The pollen grains are oblate - spheroidal (the ratio between the polar axis and the equatorial axis P/E = 0.99). Their outline in equatorial view is elliptic and in polar view is circular. The length of the colpus reaches up to 16.3 µm while its width reaches up to 10.8 µm. The distance from one colpus to another (mesocolpium) reaches up to 30 µm. The thickness of the exine is approximately 3.75 µm. Its sculpture is echinate-microechinate with verrucae.

Fig. 3.*Valeriana tuberosa* (in its habitat)

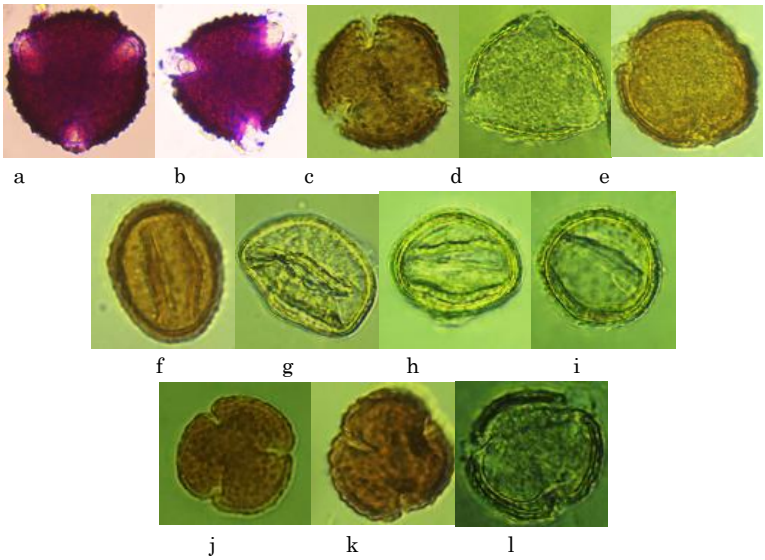

Fig. 4. Pollen grains of *Valeriana tuberosa*a, b. pollen grains in polar view (basic fuchsine method, 40x magnification); c, d, e. pollen grains in polar view x400 (acetolysis method); f, g, h, i. pollen grains in equatorial view x400 (acetolysis method); j, k, l. pollen grains in polar view x400 (acetolysis method) (photo: **Kallajxhiu, N**.)

Genus: ***Valeriana* L.**
Species: *Valeriana officinalis* L.
Valeriana officinalis is a perennial plant with a short rhizome. Its flowers are hermaphrodite, pink or white color. It blooms in the months of May - July. This plant grows in humid places and forests.The material was found in Ruen, in the Shebenik National Parkon 20. 07. 2023, Albania.

The palynomorphological study showed that the pollen grains are monads, tricolpate and isopolar. In the polar view they have a circular shape, while in the equatorial view they appear elliptical. The length of the polar axis varies from 40 - 51 (47.5) µm and the length of the equatorial axis varies from 45 - 52 (49.5) µm. Starting from the sizes of pollen grains, according to Kapidani (1996), they are classified as medium-sized (25 - 50 µm). This is in full agreement with the literature (Halbritter, H. & Auer, W., 2020). The ratio between the length of the polar axis of the pollen grain and that of the equatorial axis P/E = 0.95, so they are classified as oblate to spheroidal.

The length of the colpi is approximately 18.3 µm, while the width reaches up to 9.6 µm. The distance from one colpus to another (mesocolpium) reaches up to 30.5 µm. The exine layer reachesup to 4.5 µm. It is presented with a spiny sculpture (echinate) with spines longer than 1 µm. This feature is clearly seen in figure 6.

Fig. 5. *Valeriana officinalis* (in its habitat)

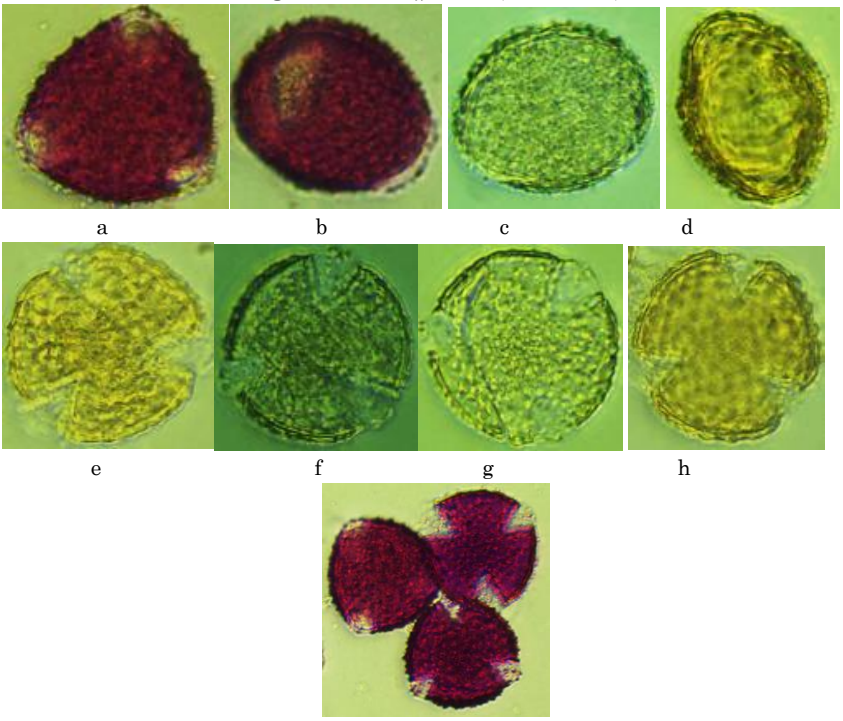

Fig. 6. Pollen grains of *Valeriana officinalis* a. pollen grains in polar view x400, b. in equatorial view x400 (basic fuchsine method); c, d. pollen grains in equatorial view x400 (acetolysis method); e, f, g, h. pollen grains in polar view x400 (acetolysis method); i. pollen grains in polar view x400 (basic fuchsine method) (photo: **Kallajxhiu, N**.)

To determine from the palynomorphological view wether there are differences between the pollen grains, the comparison of the pollen grains of the two studied species of the *Valeriana* taxa was made.

The following table shows the average compared values for the length of the polar axis, the length of the equatorial axis, the length of the colpus, its width and the distance from one colpus to the other for the two studied species. From the data in table 1, it is clear that the pollen grains of V. *officinalis* are larger in average values than those of *V. tuberosa*. Also, the length of the colpus of these grains is greater, but the

colpus of the pollen grains of *V. tuberosa* is somewhat narrower than that of *V. tuberosa*. Mesocolpium is almost equal in both pollen grains. In the pollen of *V. officinalis*, the thickness of the exine is greater than that of *V. tuberosa*. Its sculpture in the pollen grains of *V. tuberosa* is echinate-microechinate with verrucae while that of *V. officinalis* is presented with a spiny sculpture (echinate) with spines longer than 1 µm.

Table 1. The average values of the palynomorphological characteristics of the pollen grain for *V. tuberosa* and *V. officinalis*.

Pollen grain features	***Valeriana tuberosa*** **Average dimension**	***Valeriana officinalis*** **Average dimension**
Polar axis (P)	45.2 µm	47.5 µm
Equatorial axis (E)	45.5 µm	49.5 µm
The length of colpus	16.3 µm	18.3 µm
The width of colpus	10.8 µm	9.6 µm
Mesocolpium	30 µm	30.5 µm
The thickness of exine	3.75 µm	4.5 µm
P/E	0.99	0.95

Figure 7 shows graphically the comparison between the minimum and maximum values of the pollen grains of two species of the *Valeriana* genus.

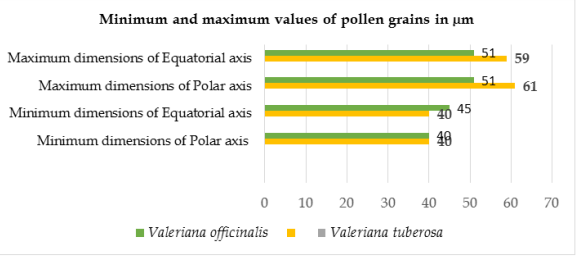

Fig. 7. Minimum and maximum values compared

CONCLUSIONS

From the palynomorphological study carried out with the species found in the National Park of Shebenik in Albania, it was found that the pollen grains of *Hyacinthella leucophaea* ssp. *atchleyi* were monocolpate and medium sized (25 - 50) µm while those in the *Valeriana* genus were tricolpateand considered medium or large - sized (25 - 50; 50 - 100) µm.

The pollen grains of *Hyacinthella leucophaea* ssp. *atchleyi* were prolate while the pollen grains of Valeriana genus were spherical in polar view and elliptical in equatorial view.

The exine layer in *Hyacinthella leucophaea* ssp. *atchleyi* were reticulate and in the pollen grains of Valeriana genus it variedfrom echinate in *Valeriana officinalis* to echinate - microechinate with verrucae in *Valeriana tuberosa*. The exine layer of the pollen grain of *Valerina officinalis* was thicker than that of *Valeriana tuberosa*.

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