

Palynomorphological study of pollen grains of three Fritillaria genus species (Liliaceae) in Albania

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Abstract. Pollen grains of three species of *Fritillaria* genus, specifically: *messanensis* subsp. *gracilis*, *pontica* and *macedonica*, were described for the first time to highlight palynomorphological features and the differences between them based on the characteristics of sporoderm and dimensions. They had monosulcate aperture and based on the P/E ratio, pollen grains were wide oval in polar view and long oval in equatorial one. The dried pollen grains appeared boat-shaped in the three species. The membrane of sulcus was variable from reticulate at *Fritillaria macedonica*, verrucate at *Fritillaria messanensis* subsp. *gracilis*, and psilate-granulate at *Fritillaria pontica*, but it went from one pole to the other only at *F. pontica* species. The results of the study showed that the exine varied from perforate at *F. messanensis* subsp. *gracilis*, reticulate-heterobrochate at *F. pontica* species, and reticulate with almost equal reticles at *F. macedonica* species. The only difference in exine sculpture between *F. pontica* and *F. macedonica* species was that brochi were larger at *F. macedonica*. The minimum dimensions for all the palynomorphological studied features were present at pollen grains of *Fritillaria macedonica*, whereas regarding the maximum dimensions, those were found at *F. pontica* species for the width of pollen grains and dimensions of sulcus and at *F. messanensis* subsp. *gracilis* species for the length of pollen grains and exine thickness.

Key words: aperture, *Fritillaria*, Liliaceae, pollen grains, sulcus.

Introduction

After Vangjeli et al. (2000), Liliaceae family is represented by 25 genera, and among them the genus *Fritillaria* counts five species. In the whole world, genus is represented by 7 subgenera and 165 taxa (139 species, 17 subspecies, and 9 varieties). Species of *Fritillaria* genus are found naturally in the most tempered areas of the Northern Hemisphere, from North America, along Europe, the Mediterranean region, and Central Asia to

China and Japan in the east (Tekşen et al., 2010). The most species in this genus belong to the main subgenus - *Fritillaria*. The Mediterranean region is the center of genetic diversity, with the most taxa described by Turkey and Iran (Zagros Mountains) as the diversity center at the species level (Hosseini, 2018).

Fritillaria macedonica is listed in Albania's National Red List of Flora and Fauna, and its status is LRnt (Lower Risk, near threatened). According to

Vangjeli et al. (1994), *Fritillaria macedonica* has been classified as rare in Albania. Shuka & Matevski (2018) have evaluated its threat status according to the new IUCN criteria (IUCN, 2012), and it was concluded that *Fritillaria macedonica* is an Endangered (EN) taxon that fulfills the criteria B1ab (i,ii,iii,v)+2ab(i,ii,iii,v) for inclusion in the IUCN threat categories.

Based on the restricted distribution area and the small number of individuals, *Fritillaria macedonica* has been classified as Vulnerable (VU) at a national level in Greece by Tsiftsis et al. (2022), whereas in Serbia the species was included in the Red Data Book of the country as Critically Endangered (CR) (Stevanović, 1999, Tomović et al., 2007).

Whereas *Fritillaria messanensis* is listed in Albania's National Red List of Flora and Fauna with the status CR A1b (Critically Endangered) under criterion A1b, indicating it faces an extremely high risk of extinction in the wild. *Fritillaria messanensis* lacks a global IUCN assessment, so its international (IUCN) conservation status is currently not evaluated.

Following the literature resources (Hosseini, 2018; Samaropoulou et al., 2021; Tekşen et al., 2010), the shape of pollen grains was symmetrical, heteropolar, prolate (elongated in the poles), or sometimes subprolate (a bit elongated, but approximately spherical). The exine of pollen grains of this genus varied from reticulate, reticulate-brochate, and reticulate accompanied by intertwined elements to psilate-brochate, while the sulcus membrane varied from psilate, verrucate, verrucate-granulate, and granulate to granulate-striate.

This article aims at assessment and highlighting of the existence of similarities and differences in the palynomorphological features of pollen grains of three species of *Fritillaria* genus.

Materials and methods

The specimens of the three species of the *Fritillaria* genus were collected fresh in Shebenik, Krastë-Elbasan and Korab.

The determination of plant species and pollen grains features description was done based on the personal experience and literature, specifically Vangjeli et al. (2000) for the plants, and Faegri & Iversen (1989), Hesse et al. (2009), Hosseini (2018), Kapidani (2005), Ozler & Pehlivan (2007), Pupuleku et al. (2014), Samaropoulou et al. (2021), and Tekşen et al. (2010) for pollen grains.

The morphological characteristics of the pollen grains were studied using three analytical methods as follows:

- Erdtman's acetolysis method (Erdtman, 1960);
- Avetisjan's simplified acetolysis method (Avetisjan, 1950);
- Smoljaninova & Gollubkova's basic fuchsin method (Smoljaninova & Gollubkova, 1953).

The first two acetolysis methods were used to obtain better results in the study of sporoderm elements, while the basic fuchsin methods were used for the study of the shape and structure of the pollen grains.

From the treatment of pollen grains of the 5 studied plants, 3-6 microscopic slides per plant were prepared and a lot of pictures were taken with the Motic Images Plus 2.0 ML B1 Series optical microscope. Microscopic pictures of pollen grains of the studied plants were taken with X400 magnification by Blerina Pupuleku and Klea Trokoliçi.

Results and Discussion

Fritillaria messanensis subsp. *gracilis* Rafin. in Derv., *Jaur. Bot. Appl.* 4: 272(1814)

Bulbous geophyte. In sparse forests and pastures. May-June. Subendemic. The material collected in Krastë-Elbasan was in the fresh conditions (Vangjeli et al., 2000).

The pollen grains were monosulcate with bilateral symmetry and prolate (elongated in poles) ($P/E = 1.39$). They were boat-shaped in their dried form. In polar position, pollen grains were wide oval, while in equatorial one, they were long oval.

The sulcus had verrucate membrane, which did not go from one pole to the other. The length of sulcus varied from 33.18 to 47.04 (41.65) μm , while its width varied from 12.6 to 32.76 (23.07) μm .

The exine was composed of two equal layers with perforate sculpture. Its thickness varied from 1.26 to 4.2 (2.44) μm . The cytoplasm was granular.

The length of pollen grains varied from 45.78 to 60.9 (50.43) μm , while the width varied from 25.2 to 40.74 (36.11) μm (Fig. 1), (Table 1).

Fritillaria pontica Wahlenb., *Isis* 21: 984 (1824) - The Pontic fritillary

Bulbous geophyte. In forest clearings and scrubs. May-June. Balkanic. The material collected in Korab was in the fresh conditions (Vangjeli et al., 2000).

The pollen grains were monosulcate with bilateral symmetry and (elongated in poles) ($P/E = 1.29$). They were boat-shaped in their dried form. In polar position, pollen grains were wide oval, while in equatorial one, they were long oval.

The sulcus had psilate-granulate membrane accompanied by ornaments and went from one pole to the other. The length of sulcus varied from 33.6 to 49.98 (42.2) μm , while its width varied from 16.8 to 34.86 (25.36) μm .

The exine was composed by two equal layers and reticulate-heterobrochate sculpture with unequal reticuli. Its thickness varied from 1.26 to 2.94 (2.22) μm . The cytoplasm appeared granular.

The length of pollen grains varied from 43.26 to 56.7 (49.76) μm , while the width varied from 29.82 to 45.78 (38.38) μm (Fig. 2), (Table 1).

***Fritillaria macedonica* Bornm., Feddes Repert. 19: 200 (1923) - The Macedonian fritillary**

Bulbous geophyte. In pastures and humid places of high mountainous areas, primarily in Eastern and Central Albania. May-July. Sub-endemic. The material collected in Shebenik was in the fresh conditions (Vangjeli et al., 2000).

The pollen grains were monosulcate with bilateral symmetry and (elongated in poles) ($P/E = 1.32$). They were boat-shaped in their dried form. In polar position, pollen grains were wide oval, while in equatorial one, they were long oval.

The sulcus had reticulate membrane, but it did not go from one pole to the other. The length of sulcus varied from 22.5 to 31.5 (26.42) μm , while its width varied from 10.5 to 18 (13.65) μm .

The exine was composed of two equal layers. Its sculpture appeared reticulate with almost equal reticuli. The thickness varied from 1.05 to 1.5 (1.41) μm . The cytoplasm appeared granular.

The length of pollen grains varied from 30 to 37.5 (33.29) μm , while the width varied from 21 to 28.5 (25.21) μm (Fig. 3), (Table 1).

The palynomorphological study of three species of *Fritillaria* genus respectively *F. messanensis* subsp. *gracilis*, *F. pontica* and *F. macedonica* accentuate some joint characteristics as follows: heteropolar monad with monosulcate aperture, wide oval shape of pollen grains in polar view and long oval shape in equatorial one, boat-shaped feature of dried pollen grains, and granular cytoplasm.

Between pollen grains of three studied species (Table 2), differences were highlighted, which consisted on:

- Sporoderm features, where the exine was variable from reticulate-heterobrochate at *F. pontica* species, reticulate with almost equal reticles at *F. macedonica* species, and perforate at *Fritillaria messanensis* subsp. *gracilis*;
- Brochi, that were larger at *F. macedonica* species;
- The membrane of sulcus, which varied from reticulate at *F. macedonica* species, verrucate at *F. messanensis* subsp. *gracilis*, and psilate-granulate at *F. pontica* one;
- Sulcus, which went from one pole to the other only at *Fritillaria pontica*.

Referred to Fig. 4, pollen grains of *Fritillaria macedonica* plant resulted to have smaller dimensions for all studied palynomorphological features.

Regarding Fig. 5, averagely smaller dimensions were found at *Fritillaria macedonica* for all palynomorphological characteristics, while averagely bigger dimensions were at *F. pontica* species for the width of pollen grains and sulcus dimensions and *F. messanensis* subsp. *gracilis* species for the length of pollen grains and exine thickness.

The maximum dimensions were present at *F. pontica* species for the width of pollen grains and sulcus dimensions, and at *F. messanensis* subsp. *gracilis* specie for the length of pollen grains and exine thickness according to the data of Fig. 6.

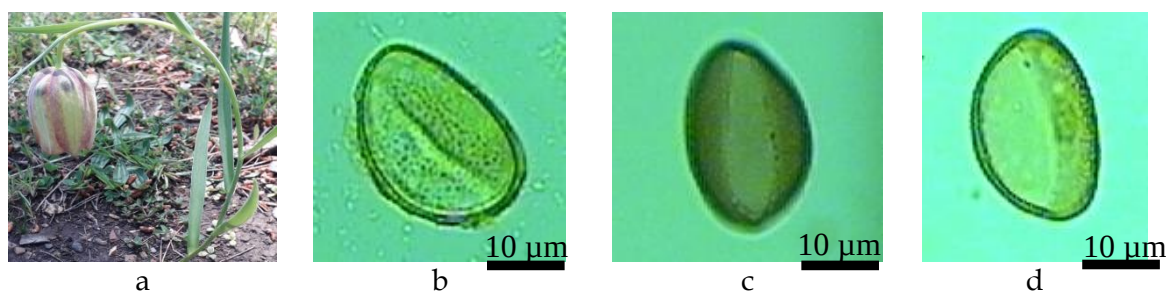


Fig. 1. a: Pollen grains of *Fritillaria messanensis* subsp. *gracilis*; b, c, d: Polar view X400.

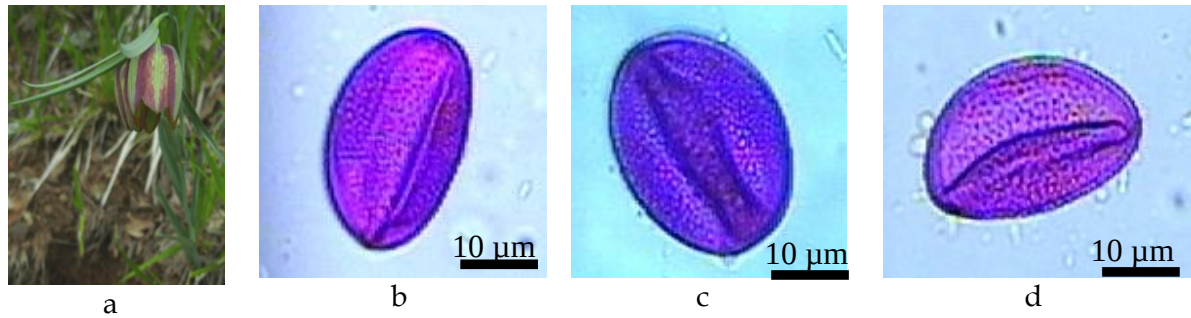


Fig. 2. a: Pollen grains of *Fritillaria pontica*; b,d: Polar view X400; c: Equatorial view X400.

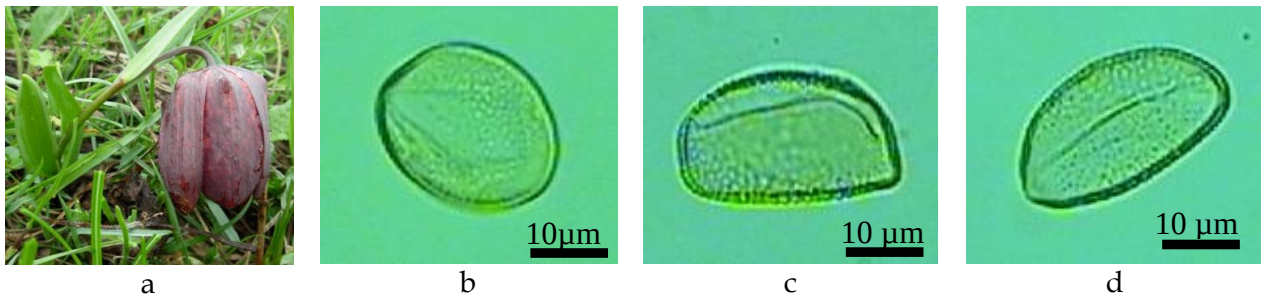


Fig. 3. a: Pollen grains of *Fritillaria macedonica*; b, c: Equatorial view X400; d: Polar view X400.

Table 1. Pollen grains' measurements of the three species of *Fritillaria* genus from Albania based on palynomorphological data.

Features Species	Values	Polar axis (P) µm	Equatorial axis (E) µm	P/E ratio	Sulcus length µm	Sulcus width µm	Exine thickness µm	Shape
<i>Fritillaria messanensis</i> subsp. <i>gracilis</i>	Minimum	45.78	25.2	1.82	33.18	12.6	1.26	Prolate
	Average	50.43	36.11	1.39	41.65	23.07	2.44	
	Maximum	60.9	40.74	1.49	47.04	32.76	4.2	
<i>Fritillaria pontica</i>	Minimum	43.26	29.82	1.45	33.6	16.8	1.26	Prolate
	Average	49.76	38.38	1.3	42.2	25.36	2.22	
	Maximum	56.7	45.78	1.24	49.98	34.86	2.94	
<i>Fritillaria macedonica</i>	Minimum	30	21	1.43	22.5	10.5	1.05	Prolate
	Average	33.29	25.21	1.32	26.42	13.65	1.41	
	Maximum	37.5	28.5	1.31	31.5	18	1.5	

Table 2. Pollen grains' palynomorphological features of the three species of *Fritillaria* genus from Albania.

Species of <i>Fritillaria</i> genus	Aperture type	Exine ornamentation	Sulcus apex	Sulcus membrane ornamentation
<i>Fritillaria messanensis</i> subsp. <i>gracilis</i>	Monosulcate	Perforate	Sharp	Verrucate
<i>Fritillaria pontica</i>	Monosulcate	Reticulate-heterobrochate	Sharp	Psilate-granulate
<i>Fritillaria macedonica</i>	Monosulcate	Reticulate with almost equal reticles	Sharp	Reticulate

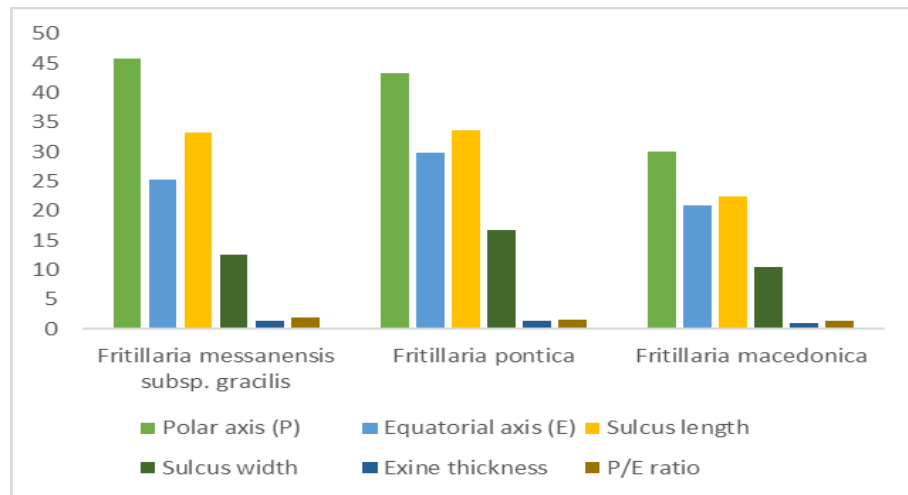


Fig. 4. Minimum dimensions of three studied species: *Fritillaria messanensis* subsp. *gracilis*, *Fritillaria pontica* and *Fritillaria macedonica*.

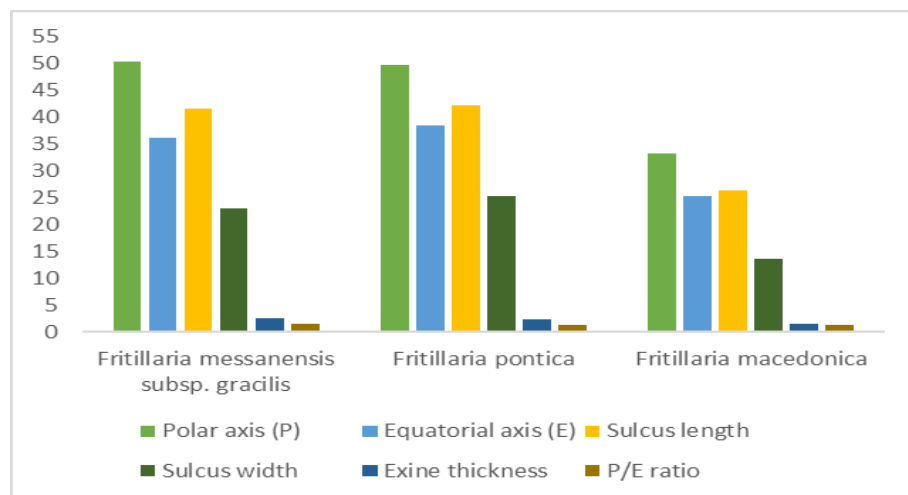


Fig. 5. Average dimensions of three studied species: *Fritillaria messanensis* subsp. *gracilis*, *Fritillaria pontica* and *Fritillaria macedonica*.

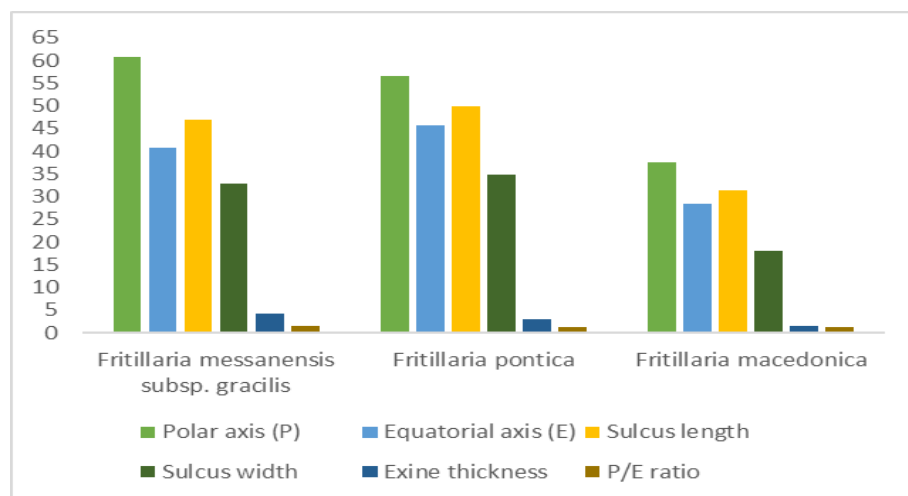


Fig. 6. Maximum dimensions of three studied species: *Fritillaria messanensis* subsp. *gracilis*, *Fritillaria pontica* and *Fritillaria macedonica*.

Conclusions

From the morphological study of pollen grains of three *Fritillaria* genus species respectively *messanensis* subsp. *gracilis*, *pontica* and *macedonica*, the following differences were distinguished:

- The exine sculpture, which varied from reticulate with larger brochi at *F. macedonica* species, reticulate-heterobrochate at *F. pontica* species, and perforate at *F. messanensis* subsp. *gracilis*;
- The sulcus membrane, which appeared reticulate at *F. macedonica* species, verrucate at *F. messanensis* subsp. *gracilis*, and psilate-granulate at *F. pontica* one.

The minimum dimensions for all studied palynomorphological characteristics were found at pollen grains of *F. macedonica* species, while the maximum dimensions were seen at *F. pontica* species for the width of pollen grains and dimensions of sulcus, and at *F. messanensis* subsp. *gracilis* species for the length of pollen grains and exine thickness.

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Received: 14.10.2025

Accepted: 09.12.2025