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PALYNOMORPHOLOGICAL STUDY OF *DIANTHUS PETRAEUS* WALDST. ET KIT. (CARYOPHYLLACEAE)

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Abstract: The pollen morphology of *Dianthus petraeus* (Caryophyllaceae), which is endemic to the Balkans, has been examined by both light and scanning electron microscopy in order to provide taxonomically valuable characters that might be used in the classification of the Caryophyllaceae, as well as to contribute to the pollen atlas of Serbian apiflora. The pollen grains of *D. petraeus* are radially symmetrical, apolar, spheroidal and medium-sized. The mean of the pollen diameter is 45.5±1.4 μm. The sculpturing pattern of exine is microechinate-microperforate, and the tectum is covered with minute echini averaging 0.8±0.1 μm in length, more or less evenly distributed between the perforations. The microechini average number per sample area of 5 μm x 5 μm is 3.2±0.6. In view of the number, position and type of the apertures, the grains are polypantoporate, with about 13 operculate pores, averaging 6.4±0.7 μm in diameter. Each operculum is covered with 4-6 conical spinules, which are longer than those on the exine surface. The obtained palynological results provide information that contribute towards a better understanding of the taxonomic status of *D. petraeus*.

Key words: pollen morphology; *Dianthus;* endemic; light microscopy; SEM

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INTRODUCTION

The genus *Dianthus* belongs to the family Caryophyllaceae (order Caryophyllales*)* which is comprised of 86 genera and 2200 species that are mainly distributed in the temperate regions of the northern hemisphere with a center in the Mediterranean and Irano-Turanian region. A number of genera (especially of the subfamily Paronychioideae) occur predominantly in arid areas, with few in higher altitudes of tropical mountains and in southern temperate regions (Bittrich, 1993). The genus *Dianthus* includes diverse annual or perennial herbaceous plants, very rarely subshrubs (*Dianthus gracilis* Sibth. et Sm.), native mainly to Europe and Asia, with a few species extending to North Africa and North America (Mabberley, 2002). They are adapted to the cooler Alpine regions of Europe and Asia, but are also found in Mediterranean coastal regions. Over 300 *Dianthus* species have been identified, of which 27 occur in Serbia (Gajić, 1970). These plants have always attracted attention, both in terms of widespread multipurpose use as well as from a scientific point of view. Some of them have been cultivated for hundreds of years for ornamental and culinary purposes, then as aromatic plants containing essential oils present in small amounts in the petals, or as medicinal herbs traditionally prescribed in European folk medicine (Ingwerson, 1949; Facciola, 1990; Hughes, 1993; Bown, 1995; McGeorge and Hammett, 2002).

Besides morphological studies on which Caryophyllaceae systematics and classification are based, many palynological studies aimed to clarify the taxonomic position of the genera within the family, as well as to support or to disprove grouping or separation of taxa within the subfamilies of Caryophyllaceae (Bittrich, 1993). There are a large number of studies devoted to pollen morphology of the family Caryo-

phyllaceae (Ghazanfar, 1984; Yildiz, 1996a,b, 2001a,b; Ataşlar et al., 2009). Some of the earliest palynological studies of this family were carried out by Faegri and Iversen (1950), Erdtman (1952) and Erdtman et al. (1961). Extensive palynomorphological investigations of some Caryophyllaceae taxa were performed in Scandinavian countries (Chanda, 1962), Ukraine (Romanova, 1992; Romanova and Bezusko, 1987), Egypt (Taia, 1994) and in Turkey (Yildiz, 2001a, b; 2005; Kaplan, 2008; Ataşlar et al., 2009). The most comprehensive and detailed comparative morphological research on Caryophyllaceae pollen focusing on the surface ultrastructure using both light and scanning electron microscopy, is that of Perveen and Qaiser (2006) on 74 Pakistani species belonging to 23 genera, followed by Al Eisawi (1989) on 32 Jordanian species belonging to 18 genera, and Punt and Hoen (1995) on northwestern European flora.

Dianthus taxa have been widely researched from a palynological standpoint. Bloch et al. (2006) contributed to palynological and pollination aspects of the genus by investigating the relationship between different pollen amounts deposited on the stigma and the resulting reproductive success in *D. carthusianorum*. Somogyi (2013) studied pollen for taxonomic evaluation of Central European *Dianthus* species of sect. *Plumaria* based on molecular evidence. Some authors, such as Sahreen et al. (2008) and Kizilpinar et al. (2010), focused their research on the pollen morphological features of selected *Dianthus* species growing in the countries they come from. In addition, Yildiz et al. (2009) studied the pollen of *Dianthus cyprius* within an extensive palynological study of 19 endemic Caryophyllaceae taxa from Northern Cyprus. Yildiz (2001b) himself investigated 45 Turkish Caryophyllaceae, including ten *Dianthus* species. Chanda (1962) described the pollen morphology of *Dianthus deltoides* within an investigation of 36 species, varieties and hybrids from nine genera of Caryophyllaceae.

Dianthus petraeus Waldst. et Kit. (Caryophyllaceae) is a Central Balkan endemic species belonging to the sub-Moesian floral element (Gajić, 1984). In Serbia, this perennial hemicryptophyte is widespread in chasmophytic vegetation, most commonly inhab-

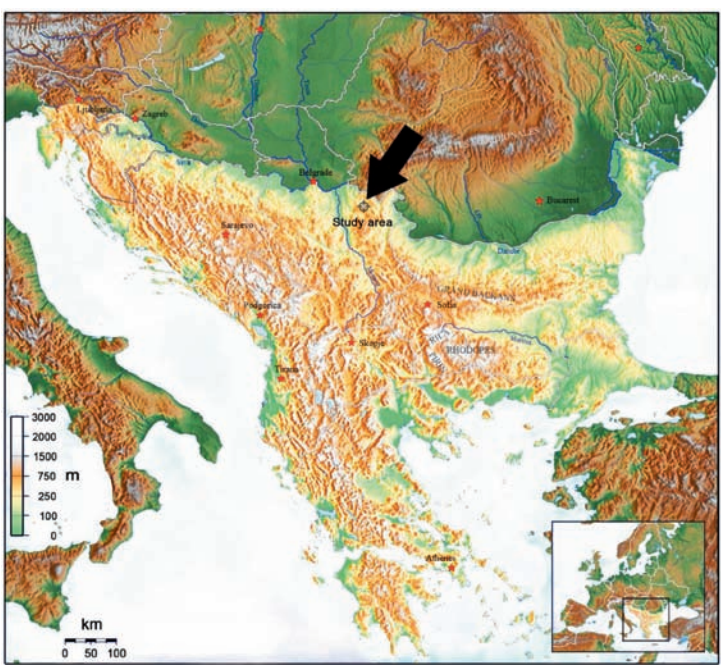

Fig. 1. Map of the Balkans indicating the study area (modified from the website: http://sr.wikipedia.org/wiki/).

iting calcareous rocky slopes, and it also occurs at the edge of xerophilous forests of highland regions and within some mountain phytocoenoses (Gajić, 1970; Kojić et al., 1997) (Fig 1). Based on the flower morphological features (color of the corolla, division depth of featherlike petals, calyx tube length, number of epicalyx scales, flower fragrance), as well as the leaf morphology, the following taxa have been listed inside the Serbian region: *D. petraeus*. ssp. *noeanus*, *D. petraeus*. ssp. *integer*, *D. petraeus*. ssp. *petraeus*, *D. petraeus* ssp. *petraeus* f. *petraeus* and *D. petraeus* ssp. *petraeus* f. *liliodorus*).

There are no previous reports dealing with the pollen morphology of *D. petraeus* that the present research is based on. The aim of the current study was to provide palynological information about this endemic species, and thereby contribute to the completion of the pollen atlas of Serbian apiflora, as well as to melissopalynological and taxonomic investigations. A description of the pollen characteristics of this taxon may be helpful for identification and systematics, and may contribute to a better understanding of phylogenetic relationships within the Caryophyllaceae. Taking into account all the studies of previously palynomorphologically described *Dianthus* taxa, the current study also intended to clarify the taxonomic status of *D. petraeus*.

MATERIALS AND METHODS

Study species

A field survey was conducted in a natural population of *Dianthus petraeus* growing on a south-facing rocky slope in the gorges of the Crna Reka and Tisnica Rivers (eastern Serbia) during the vegetation period of 2013. Flower specimens and data on the distribution and flowering phenology were collected. Pollen material was obtained from flowers at full flowering stage, taken from plants grown at an altitude of 450-550 m a.s.l. *D. petraeus* was sporadically present at the study site during the observation period, which lasted from June until late August 2013. The Flora of Serbia (Gajić, 1970) was used for species identification.

Plants, growing to 30 cm in height, produce solitary, white, many-stellate protandrous flowers (Fig 2). The feathery divided petals are enclosed in a narrow calyx tube and end in a flat rim, which serves as a landing platform for insect pollinators. The calyx is tube-like, with short epicalyx scales at the base. The pollination observation data support the presumption that, like in some other *Dianthus* species, the strongly scented flowers, light-colored feather-like petals and long calyx tube evolved as a result of adaptation to nocturnal pollinators (moths and hawk moths) (Knuth, 2013; Somogyi, 2013).

Research area

Plant specimens were found in the chasmophytic vegetation of the gorges of the Crna Reka and Tisnica, located in the hilly-mountainous part of eastern Serbia (Carpathian-Balkan region, territory of Braničevski County) (Fig.1). Eastern Serbia is characterized by sharp and cold winters with strong and frequent winds, large snowdrifts and a very pronounced long, dry period in the middle of the growing season. However, in the studied area, cooler air descends from the higher parts of the slope to the bottom of the gorge, modifying the climate, which has led to the inversion of vegetation, resulting in

Fig. 2. *Dianthus petraeus* growing on a rocky slope in the Tisnica River gorge.

mesophilic plants in the lower part of the canyon and thermophilic plant communities at higher elevations (Mišić, 1981).

Light and scanning electron microscopy

Pollen morphology of *D. petraeus* was examined by both light microscopy (LM) and scanning electron microscopy (SEM). The following features describing pollen grains were examined: symmetry, polarity, size and shape, spinule and perforation size and density, number, position and diameter of pores, the interporal distance, operculum ornamentation and exine thickness.

For the LM study, pollen grains from mature anthers were mounted in Kaiser's glycerin jelly. Observations were made with a Leica DMSL microscope equipped with a digital camera (Leica DC 300) and Leica IM1000 software. The general shape, number of pores and exine thickness were defined and measured under LM. Data on these parameters are based on the measurements of 50 pollen grains.

For the SEM study, samples were mounted directly on metallic stubs using double-sided adhesive tape and coated with gold (in BAL-TEC SCD 005 Sputter Coater, 100 seconds in 30 mA) in a sputtering chamber. The detailed surface ornamentation and the aperture characteristics were examined using a JEOL JSM-6390 LV electron microscope at an acceleration voltage of 20 kV. SEM micrographs were used mainly for examining the shape and diameter of grains, number, position and diameter of pores, as well as to obtain more detailed information on the ornamentation related to ground sculpture, size and density of spinules and the interporal distance. Observations and measurements were done on a sample of 30 or more grains for each morphological character, and arithmetic means and standard deviations were calculated.

RESULTS

The pollen grains of *Dianthus petraeus* are monads, radially symmetrical, apolar, spheroidal and medium-sized (Figs. 4 a, b). LM analyses revealed circular outlines (Fig. 3). The mean of the pollen diameter is 45.5±1.4 μm. The exine, measured under LM, is on average 3.7±0.4 μm thick and tectate. The sculpturing pattern of the exine is microechinate-microperforate. SEM revealed a clearly visible microperforate tectum covered with smooth and predominantly obtuse supratectal spinules (minute echini) averaging 0.8±0.1 μm in length, more or less evenly distributed between the perforations (Figs. 4 c, d). Actually, these tectal sculptural elements are more like very small cones that taper smoothly from a flat circular or roughly circular base to the apex lying anywhere outside the plane of the base. The microechini density per sample area of 5 μm x 5 μm averages 3.2±0.6. The perforations of exine surface are rather sparse, elliptic to circular, averaging 0.6±0.2 μm in diameter. The number of microperforations per sample area (25 μm^2) ranges from 0 to 2.

Concerning the type, number and position of the apertures, grains are found to be polypantoporate, with pores distributed more or less regularly over the whole surface. The pore number varies between 12 and 14. Pores average 6.4±0.7 μm in diameter, and are circular and operculate. Each operculum is on average 1.5±0.05 μm high, covered with 4-6 conical spinules 1.4±0.2 μm in length, which are longer than those on the exine surface. The mean interporal distance is 13.3±1.3 μm.

DISCUSSION

The morphological similarities of pollen grains among Caryophyllaceae taxa relate to general characters such as polarity, symmetry and shape; on the other hand, features such as the size class, type, number and position of apertures as well as the sculpturing pattern and thickness of exine, due to considerable variation, are found to be of taxonomic value and useful in species delimitation (Perveen and Qaiser, 2006; Sahreen et al., 2008). Pollen grains of the majority of Caryophyllaceae are spheroidal, apolar (rarely isopolar) and radially symmetrical which is in accordance with the pollen description in the present study. Except for the prevailing spheroidal grains of species classified in *Silene indica*-type and *Stellaria media*-type (including *Dianthus* species), Perveen and Qaiser (2006) found also prolate-spheroidal (*Spergula*

arvensis, S. marina, S. media, Polycarpaea corymbosa, P. spicata), sub-prolate (*Spergula fallax*) to rarely prolate grains (*Telephium imperati*). Although different species were investigated, Al Eisawi (1989) agreed that the shape is mostly spheroidal and rarely prolate or polyhedral with hexagonal to octagonal outline, while Ataşlar et al. (2009), reported that grains may be suboblate to subprolate (if 3-colpate) and spherical or rounded polyhedral (if porate or pantocolpate).

Apart from a few exceptions (*Petrorhagia alpina, Agrostemma githago*), previous studies have demonstrated that Caryophyllaceae pollen is usually of medium size, ranging from 25 to 50 (65) µm in diameter (Yildiz, 2001a, b; Perveen and Qaiser 2006; Ataşlar et al., 2009; Yildiz et al., 2010; Poyraz and Ataşlar, 2010). Ataşlar et al., (2009) specified that grains range from 24 to 65 µm in diameter if pantocolpate and pantoporate, and from 12.5 x 8 µm to 28 x 23 µm if tricolpate or triporate. By comparing the pollen size of ten *Dianthus* taxa growing in Turkey, Yildiz (2001b) reported the smallest grain in *Dianthus leptopetalus* (34.69±2.59 µm), and the largest one in *Dianthus crinitus* var. *crinitus* (45.35±3.57 µm), which is almost of identical size as reported for *D. petraeus.* In addition, by palynological examining of the Pakistani flora, Perveen and Qaiser (2006) registered the smallest grain in *D. angulatus* (35.71±0.60 µm) and the largest one in *D. cachmericus* (50.38±1.87 µm). Within the pantoporate pollen group, Taia (1994) described two *Dianthus* species (*D. cyri* and *D. strictus*) belonging to *Vaccaria pyramidata* type (subfamily Silenoideae and tribe Diantheae), both of medium size, over 40 µm in diameter.

According to Yildiz (2001b), the general sculpturing pattern in Caryophyllaceae pollen is mainly microechinate-microperforate corresponding with the spinulose-punctate tectum, as indicated by Perveen and Qaiser (2006). Based on the ground sculpture (tectum surface) of 45 Caryophyllaceae species, Yildiz (2001b) specified four pollen groups as follows: microperforate, perforate, semireticulate and reticulate, of which microperforate are prevalent (41). There is great variation in this respect, even within a single genus. Thus, out of ten examined *Dianthus* taxa, only *D. leptopetalus* pos-

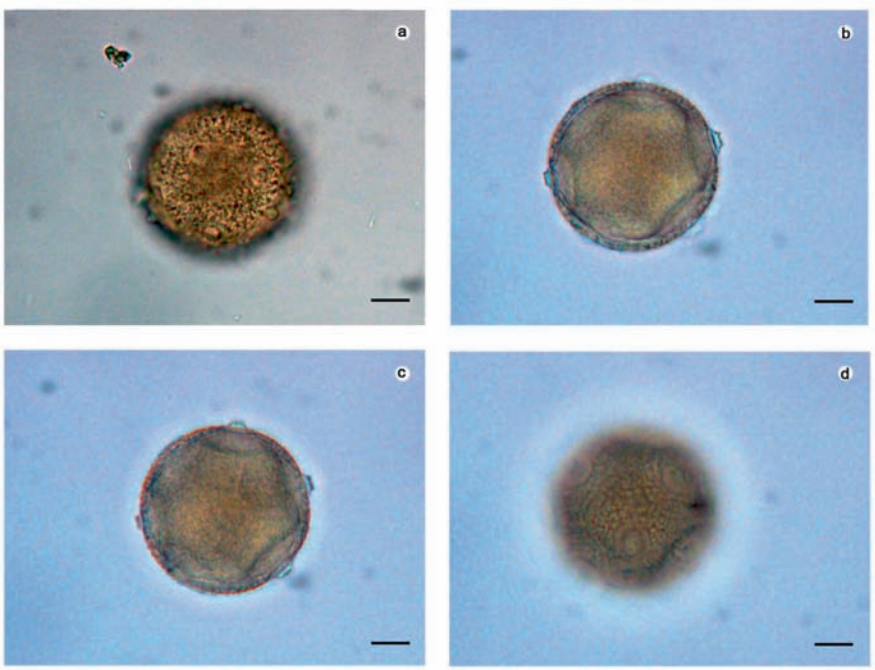

Fig. 3. Light photomicrographs of pollen grains of *Dianthus petraeus* showing circular outline, pores and exine. a, d) Surface view; c, b) Optical section.

sesses a reticulate tectum, while all others (with the exception of *D. crinitus* ssp. *crinitus* characterized as perforate) are microperforate, which is in agreement with the findings of the present study. A rarer type of ground sculpture denoted as reticulate along with microechinate supratectal elements was also described by Prentice (1987) in 130 specimens of *Silene latifolia* growing in geographically different regions in Europe and Asia. On the other hand, Perveen and Qaiser (2006) described exine ornamentation types in Caryophyllaceae as follows: spinulose-punctate, scabrate-punctate, reticulate or reticulate-scabrate. According to these two authors, the majority of species have porate pollen with spinulose-punctate tectum including all six analyzed *Dianthus* species, which corresponds to the microechinate-microperforate ornamentation found in the polypantoporate grains of *D. petraeus*.

As mentioned much earlier (Walker, 1974a, b), imperforate exine are generally regarded as primitive characteristic. Yildiz (2001b) noted a correlation between tectum surface and phylogenetic status of the species. Hence, the semireticulate and reticulate sexine found in *Dianthus leptopetalus, Silene caryophylloides* subsp. *subulata, S. conica* and *S. conoidea* indicate that they are phylogenetically more advanced than other investigated species having a microperforate tectum.

Polypantoporate pollen found in *Dianthus petraeus* is the most common aperturation type within Caryophyllaceae (Taia, 1994; Moore et al., 1997). In support of this, by examining 38 Caryophyllaceae species from the Nordic Quebec adjacent territories and from the Canadian arctic archipelago, Parent and Richard (1993) defined 13 pollen forms, 11 of which are pantoporate and the remaining two are colpate. Taia (1994) differrentiated four pollen types within Egyptian Caryophyllaceae, involving numerous subgroups: pantoporate (five types), trizonocolpate (two types), pancolpate (one type) and mixed aperturate (one type relating to one species of *Spergularia*). He found that the trizonocolpate type occurs more frequently within the subfamily Paronychioideae and sometimes in Alsinoideae, but never

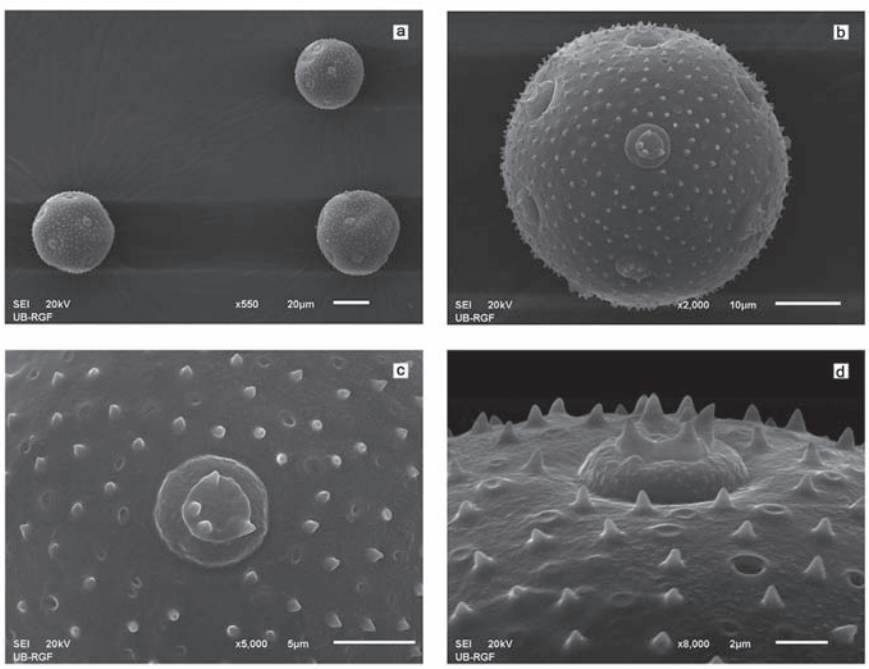

Fig. 4. Scanning electron photomicrographs: a, b) General view of polypantoporate pollen grains of *Dianthus petraeus* showing microechinate – microperforate ornamentation. c, d) Detail of exine surface showing supratectal spinules, perforations and circular pores covered with spinulate operculum.

in Silenoideae. On the other hand, pantoporate grains are rare in Paronychioidae (characterized by pancolpate type occurring in tribe Sperguleae), but are common in Alsinoideae (12-40 pores) and Caryophylloidae (15-38 pores) (Ataşlar et al., 2009). Perveen and Qaiser (2006), recognized three pollen types based on apertural description, including tectum characteristics as follows: 3(4-10)-colpate (*Spergula arvensis*-type), pantoporate with spinulose and tubuliferous/punctate ektexine (*Stellaria media*-type) and porate with reticulate ektexine (*Silene indica*-type), whereby all six analyzed *Dianthus* species (*D. anatolicus*, *D. crinitus*, *D. orientalis*, *D. angulatus*, *D. cachmericus, D. jacquemontii*) belong to the second type. Moreover, some species, such as *Spergularia marina,* showed polymorphic grains with apertures varying from hexapantocolpate to polypantocolpate or spiral (Al-Eisawi, 1989).

Polypantoporate pollen grains show a great variation in pore diameter and number. Yildiz (2001b) found that in species bearing pores with spinulate operculum, pore number varies considerably, ranging from nine (*Minuartia verna*) to 42 (*Agrostemma githago*). Comparatively analyzing ten *Dianthus* species, he reported that the number of pores varies from 10 to 20, whereby the average minimum is registered in *D. leptopetalus*, and the maximum in endemic *D. balansae*. The pore diameter in the last mentioned study ranging from 4.48±0.62 to 6.61±1.23 μm, with the 6μm size average corresponding to *D. petraeus*, was found in *D. balansae*, *D. crinitus* var. *crinitus* and *D. calocephalus*. Moreover, the author observed that the larger the pollen diameter is, the larger pore diameter and distance between pores are (with a few exceptions depending on pore number). Thus, *D. crinitus* var. *crinitus* has on average the largest pollen grain with the largest pores and interporal distance, similar to *D. petraeus.* The number of pores among *Dianthus* species is also variable; thus, for example, Sahreen et al. (2008) reported that seven *Dianthus* species from Pakistan have between 6 and 11 pores, Taia (1994) pointed that in both, *D. cyri* and *D. strictus*, 8-porate pollen is present, whereas Vural (2008) revealed in *Dianthus aytachii*, a new species from Turkey, grains with 8-14 pores. While the present study observed a pore number varying from 12 to 14, similar results have been reported by Yildiz, (2001b) for *D. carmelitarum, D. carthusianorum* and *D. calocephalus* possessing approximately 11-15 pores.

Walker (1974a, b) observed that the number of pores positively correlated with the evolutionary progress of a species. In accordance with this statement, Yildiz (2001b) believed that, due to a large number of pores, *Silene* and *Agrostemma* are more evolutionarily advanced than *Dianthus* species and genera with smaller pore numbers, such as *Arenaria, Minuartia, Stellaria, Myosoton, Cerastium* and *Moenchia.*

In this work we wished to establish phylogenetic relationships and identify interspecific differences in order obtain a better understanding of the taxonomic status of this endemic Balkan species. This study is part of a broader palynological research into all *Dianthus* species growing in Serbia. In subsequent research, the palynological variations between species and subspecies, as well as among populations of the same species or subspecies inhabiting different localities, and their taxonomical significance, will be explored. It would also be of interest to establish whether the obtained palynological results support classification based on plant morphology.

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