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Marina P. MAČUKANOVIĆ-JOCIĆ[1]*, *Snežana V. JARIĆ*[2]

[1] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Belgrade, Serbia
[2] Department of Ecology, Institute for Biological Research 'Siniša Stanković', University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia

POLLEN MORPHOLOGY OF THE BALKAN-CARPATHIAN ENDEMIC *Campanula lingulata* Waldst. & Kit. (Campanulaceae)

ABSTRACT: Palynomorphological characteristics of *Campanula lingulata*, the Balkan-Carpathian endemic species growing in Serbia, have been investigated using light microscopy and scanning electron microscopy for the first time, in order to provide some information helpful for a better understanding of the taxonomic position of this species within the genus, as well as to contribute to the pollen atlas of Serbian apiflora. The pollen grains are radially symmetrical, isopolar, 3-zonoporate and medium-sized monads oblate-sphaeroidal in shape. Mean of the polar axis (P) is 27.6±1.9 µm, while the average length of the equatorial axis (E) is 28.8±1.6 µm. The apertures are operculate. The sculpturing pattern of the exine is microreticulate–microechinatae. The exine surface is covered with evenly distributed supratectal spinules of variable length and sparse granules. The longest supratectal spinules are 0.64±0.05 µm in length and the smallest sculptural elements are less than 0.2 µm high. The microechinae density per sample area of 5 µm x 5 µm averages 17.4±2.4.

KEYWORDS: pollen morphology, *Campanula lingulata*

INTRODUCTION

Genus *Campanula* (Campanulaceae), comprising about 420 species worldwide, is distributed almost throughout the temperate regions of the Northern Hemisphere, with the greatest diversity in the Mediterranean region, whereby the distribution area extends to the Caucasus [Lammers 2007; Khansari 2012]. Centers of endemism are found in the Eastern Mediterranean, the Balkan Peninsula, the Caucasus and Turkey [Borsch *et al*., 2009]. Twenty-eight species of *Campanula* occur in the Serbian flora inhabiting various habitats such as calcareous grassy and stony places, roadsides, field margins and woodland

* Corresponding author E-mail: marmajo@agrif.bg.ac.rs

edges, from dry rocky slopes of river gorges to meadows and pastures at high altitudes in hilly and mountain areas [Obradović 1974].

Campanula lingulata Waldst. & Kit. (section *Campanula*; Campanulaceae) is the Balkan-Carpathian endemic species [Škondrić *et al*., 2014], which grows in Serbia most commonly in gorges, on rocky slopes exposed to the sun, in some sunlit shrubby habitats, and eroded terrains. For the purpose of the present study, plant specimens were collected in the gorge of the Tišnica River located in eastern Serbia. This locality was found to be very interesting from the aspect of biodiversity and the presence of relic and endemic species [Gajić 1985]. *C. lingulata* is categorized as protected in Serbia (Official Gazette of the Republic of Serbia, No. 35/10).

The earliest extensive palynological studies of the family Campanulaceae were carried out by Erdtman [1952, 1969] and Chapman [1967]. Avetisjan [1967, 1973] studied pollen morphology of this and closely related families from the aspect of taxonomy and phylogeny and also discussed an evolutionary trend in the development of apertures within Armenian Campanulales. The palynomorphological studies of different Campanulaceae species were conducted in India [Sahay 1969], Pakistan [Perveen and Qaiser 1999], and Turkey [İnceoğlu 1975, 1976; Oybak and Pınar 1995; Ocak 2003; Akçiçek *et al*., 2005; Erkara *et al*., 2008; Alçitepe 2012]. In Iran, variability and possible systematic implication of various palynomorphological characters of *Campanula* and allied genera were examined by Khansari *et al*. [2012]. Detailed palynomorphological researches on Campanulaceae, focusing on the surface ultrastructure using scanning and transmission electron microscopy, were performed by Dunbar [1973, 1975a,b, 1981, 1984].

Given that there are no previous reports dealing with the pollen morphology of *C. lingulata*, the present study represents an integral part of the extensive palynological studies, aiming at possibly providing some new information, which would be helpful for assessing the taxonomic position of this Balkan-Carpathian endemic species within the genus, as well as to contribute to the pollen atlas of Serbian apiflora.

MATERIALS AND METHODS

Research area. Plant specimens were found in the chasmophytic vegetation of the Tišnica River gorge located in temperate-continental climate area of eastern Serbia (Figure 1). Specific orography of the gorge also modifies local hygro-thermal conditions [Mišić 1981]. These modifications involve the formation of a strong thermal gradient in a small area, increase of relative humidity, frequent formation of haze, and attenuation of hygro-thermal extremes. Such climate conditions enabled protection and development of endemo-relict plants.

Study species. C. lingulata was recorded at the forest edges, in the shrub vegetation zone, as well as on a south-facing rocky slope of the gorge exposed to the sun, growing at the altitude of 450–500 m. The flower specimens of *C. lingulata* and data on the distribution and flowering phenology were collected during the vegetation period of 2013. Pollen material was obtained from

the flowers at full flowering stage from 10 plants of wild populations. *Flora of Serbia* [Obradović 1974] was used for species identification and the voucher specimens were deposited in the herbarium of the Institute for Biological Research "Siniša Stanković" in Belgrade.

During the flowering period (May–June) plants produce an inflorescence in terminal dense few-flowered heads. The pollen-collecting hairs are present on the style of protandrous flowers and on the distal surface of the stigmatic lobes. Hairs serve as a secondary pollen presentation mechanism, facilitating the transfer of pollen from the flower to insect pollinators while seeking nectar produced by floral nectaries at the top of the ovary [Nyman 1993].

Light and scanning electron microscopy. The pollen morphology of *C. lingulata* was examined by both light microscopy (LM) and scanning electron microscopy (SEM). For LM study, the pollen grains from mature anthers, prepared according to the standard acetolysis method (Erdtman 1952), were mounted in glycerine jelly and observed with a Leica DMSL microscope equipped with a digital camera (Leica DC 300) and Leica IM1000 software. The number of pores was determined and exine thickness was measured under LM. For SEM study the 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 tectal sculptural elements and the aperture characteristics were examined using JEOL JSM-6390 LV electron microscope at an acceleration voltage of 20 kV. SEM micrographs were used mainly for studing the shape and size of grains, the length of polar (P) and equatorial axis (E), number, position and diameter of pores and the interporal distance, as well as for getting more detailed information on the ornamentation referring to ground sculpture and spinule size and density. The pollen grains were photographed in polar and equatorial views, and observations and measurements were performed on a sample of 50 grains for each morphological character. The terminology used for pollen description is in accordance with Erdtman [1952] and Punt *et al.* [2007].

RESULTS

The pollen morphological features of the species examined are shown in Figure 2. a-g. The pollen grains of *C. lingulata* are shed as monads and are radially symmetrical and isopolar. The aperture type is 3-zonoporate. Equatorially distributed round operculate pores average 4.9±0.4 μm in diameter. The mean distance between two pores is 21.6±0.2 μm. The average operculum height is 2.1±0.05 μm and it had a rough, non-ornamented surface. The grains are medium-sized. Mean of the polar axis length (P) is 27.6±1.9 μm, while the length of the equatorial axis (E) averages 28,8±1.6 μm. The average P/E ratio (shape index) is 0.96±0.04, which defines the shape as oblate-sphaeroidal. In LM analyses, the outline is circular in the polar view and elliptic in the equatorial view (Figure 2. a–d). The mean exine thickness apart from microechini as measured under light microscopy is 0.9±0.1 μm.

The sculpturing pattern of exine is microreticulate-microechinate. SEM micrographs reveal clearly visible microreticulate ground sculpture of the exine covered with predominant supratectal spinules of variable size and sparse granules, more or less evenly distributed (Figure 2. g). Supratectal spinules are smooth and obtuse but sometimes with curved apices. The longest supratectal spinules are 0.64±0.05 μm in length and the smallest sculptural elements are less than 0.2 μm high. The microechinae density (spinules of all sizes) per sample area of 5 μm x 5 μm is in average 17.4±2.4.

DISCUSSION

The results of the present study, regarding the basic palynomorphological characteristics such as polarity, symmetry, size, shape, ornamentation and apertures, are completely or partially in accordance with previous palynomorphological investigations of some *Campanula* species [Nowicke *et al*., 1992; Erkara *et al*., 2008; Khansari *et al*., 2012]. The most common shape of pollen grains in Campanuloideae was reported as oblate-spheroidal [Dunbar 1975a; Erkara *et al*., 2008]. However, in subfamilies Lobelioideae [Price and Ayers 2008] and Cyphioideae the prolate shape type is present [Dunbar 1975b]. According to Erkara *et al*. [2008], who presented detailed palynomorphological features of 12 Turkish *Campanula* taxa, pollen grains are triporate (and/or tetraporate) and more or less oblato-sphaeroidal, which agrees with the current study. The oblato-sphaeroidal shape of pollen grains found in *C. lingulata,* as well as in the majority of *Campanula* species analyzed previously, is confirmed by the shape index (P/E) which is a constant feature for this and other studied Campanulaceae genera [Erkara 2008; Khansari *et al*., 2012]. As pointed out elsewhere [Khansari *et al*., 2012], the shape character within this family is not enough variable to provide a reliable feature for diagnosing supraspecific taxa at any level. The shape index for the majority of previously investigated taxa ranged from 0.92 to 0.97 [Erkara *et al*., 2008], or from 0.88 to 0.99 [Khansari *et al*., 2012] being slightly over 1.00 only in three species (*C. patula, C. phyctidocalyx, C. humilima*). Among 47 taxa examined by the latter authors, larger pollen grains (E > 40 μm) were recorded in five taxa of *Asyneuma* and six taxa of *Campanula*. In the latter study, the pollen grain size of *C. kermanica* (P-27.84±1.93 μm; E- 29.40±1.78 μm) are mostly in accordance with polar and equatorial axis measurements in *C. lingulata*.

The ornamentation in Campanuloideae is basically defined as rugulate-echinate or according to another terminology as rugulate-spinulose/verrucose [Dunbar 1975a; Perveen and Qaiser 1999]. According to Erkara *et al*. [2008] characterized the exine sculpture of 12 examined Turkish taxa of *Campanula* was described as granulate-scabrate or rugulate-scabrate and microechinate, whereas Khansari *et al*. [2012] reported that most of 47 analyzed taxa of Campanulaceae including 35 taxa of *Campanula* is characterized by rugulate-echinate or rugulate-microechinate type of ornamentation. Concerning this, the findings of the current study related to microreticulate and microechinate sculpturing

pattern of *C. lingulata* are more in accordance with those of Khansari *et al.* [2012]. Moreover, they completely correspond to those of Nowicke *et al.* [1992], who defined microreticulate tectum in *C. americana*, a species native to eastern and central North America.

The present results pertaining to the sculptural represented by spinules are in agreement with those reported by Perveen and Qaiser [1999] and Khansari *et al.* [2012], but appear contradict previous results of İnceoğlu [1975, 1976], who referred granulate sculpturing pattern. According to Khansari *et al.* [2012], the most valuable palynomorphological characters for subgeneric classification of *Campanula* are the size and density of echinae. According to the mentioned authors, the taxa in sect. *Campanula* are characterized by have the longest echinae of all analyzed species (1.00–2.10 μm) but distributed with low density (3–7/25 μm^2). Authors also found that the increase in the number of echini per unit area (5 μm x 5 μm) causes the reduction of their length. Thus, for example, *C. sclerotricha* has the lowest echini density (2 per unit area) of the maximum length (2.10 μm), and in *C. erinus* the situation is reversed (65; 0.1 μm). Also, in *C. incanescens* and *C. strigosa* was determined the same number of echini (17) per sample area (5 μm x 5 μm) as in *C. lingulata*. The microechine length in *C. lingulata* corresponds to those found in *C. lamondiae* and *C. lourica* defined by Khansari *et al.* [2012].

It has been reported that some aperture features and exine structures are among the essential criteria for determination of the phylogenetic relationships of *Campanula* species [Kuprianova 1967; Cronquist 1968; Walker 1974a,b; Takhtajan 1980]. Based on earlier studies [Dunbar 1984], all three major aperture types in Campanulaceae exist: colpate, colporate and porate and the basic number of apertures is three. According to Nowicke *et al.* [1992], only five species of *Campanula* have pantoporate polen grains (*C. americana, C. californica, C. exigua, C. griffinii* and *C. sharsmithiae*) and all other species examined have 3–4(7) pores distributed equidistantly and equatorially. However, their research revealed that in some cases, pores are unevenly distributed like in zonoporate grains of *C. angustifolia*, or they are not always uniform in size or distribution as in the pantoporate grains of *C. californica*. The results of the present study related to type, number and position of apertures corresponds to those of some Turkish *Campanula* species studied by Erkara *et al.* [2008], or some Iranian species examined by Khansari *et al.* [2012], who reported that all studied members of sect. *Rupestres* (Boiss.) possessed triporate pollen grains, while in sect. *Campanula apart from* triporate (*C. bononiensis* and *C. latifolia*), tetra- to pentaporate grains (*C. rapunculoides* and *C. trachelium)* also occurred. The latter authors pointed out that, since pore number can be variable even among different individuals of certain species, it was not a reliable character for infrageneric classification of *Campanula* and its allies.

The similarity of the pollen morphology between *C. lingulata*, the Balkan--Carpathian endemic species growing in Serbia, and *C. americana*, tne American endemic, in terms of ground sculpture of the exine, and on the other hand, some European and Asian *Campanula* species in terms of the type and number of apertures, indicates that from palynological standpoint, the taxonomic status

of the species examined might be reconsidered. The present study represents a significant contribution to the pollen atlas of Serbian apiflora, providing valuable palynological information useful in the upcoming taxonomic investigations of *Campanula* and allied genera.

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Figure 1. Map of Serbia showing the study area

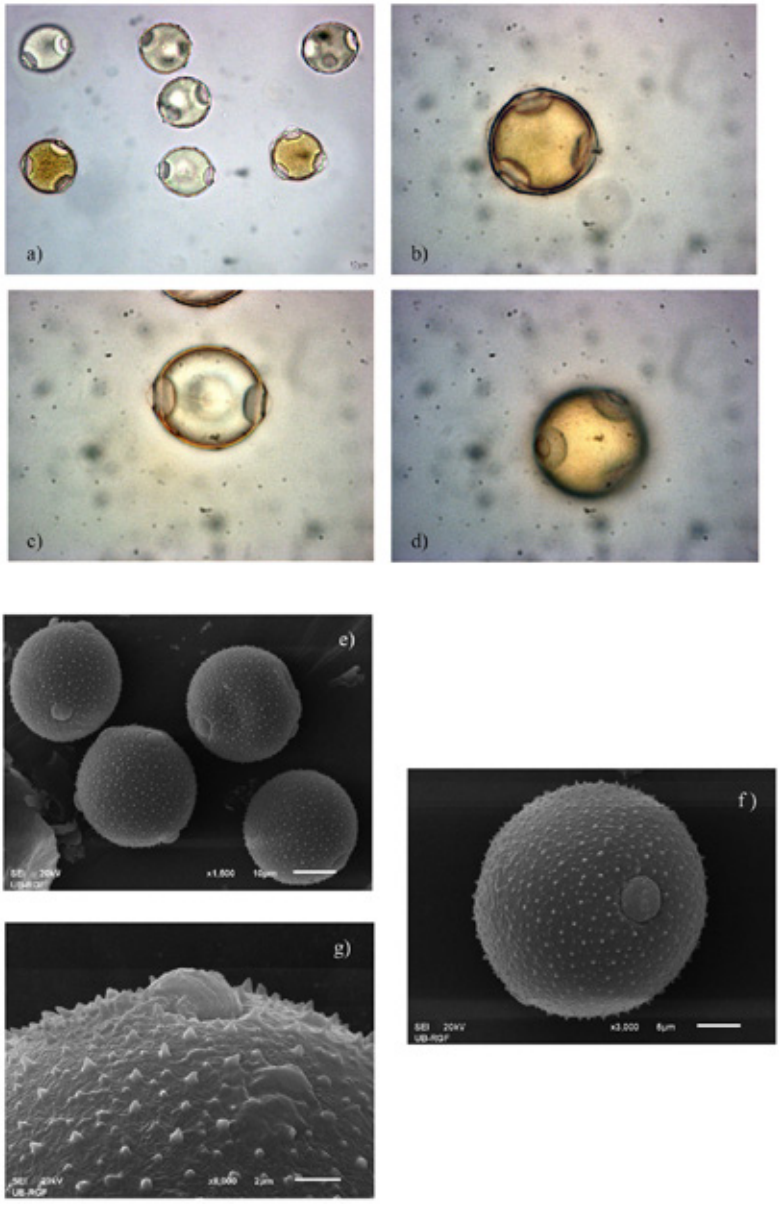

Figure 2. 3-zonoporate pollen grains of *Campanula lingulata*:
a-d) Light photomicrographs: a) general view; b) polar view in the meridional optical section; c) equatorial view; d) surface view.
e-g) Scanning electron photomicrographs: e) general view showing oblate-spheroidal pollen with porate apertures placed equidistant on the equator of the grains; f) equatorial view of pollen grain possessing sparse granules and supratectal spinules on exine surface; g) detail of exine surface showing microreticulate ground pattern of exine and circular pore covered with operculum.

МОРФОЛОГИЈА ПОЛЕНА БАЛКАНСКО-КАРПАТСКОГ ЕНДЕМИТА *Campanula lingulata* WALDST. & KIT. (Campanulaceae)

Марина П. МАЧУКАНОВИЋ ЈОЦИЋ[1] *, Снежана В. ЈАРИЋ*[2]

[1] Универзитет у Београду, Пољопривредни факултет,
Немањина 6, 11080 Земун, Београд, Србија
[2] Универзитет у Београду, Институт за биолошка истраживања
„Синиша Станковић”,
Булевар деспота Стефана 142, 11060 Београд, Србија

РЕЗИМЕ: Палиноморфолошке карактеристике *Campanula lingulata*, балканско-карпатске ендемичне врсте проучене су уз помоћ светлосне и скенирајуће електронске микроскопије, у циљу бољег разумевања таксономске позиције врсте унутар рода, као и доприноса атласу полена апифлоре Србије. Поленова зрна су средње величине, облатно-сфероидног облика, радијално симетрична, изополарна и 3-зонопоратна. На порама се уочава оперкулум. Просечна дужина поларне осе (P) износи 27,6±1,9 μm, а екваторијалне (E) 28,8±1,6 μm. Површина тектума је микроретикулатна са микроехинатном орнаментацијом. Површина егзине је прекривена равномерно распоређеним супратекталним спинулама варијабилне дужине, као и ретким гранулама. Најдужи супратектални елементи износе 0,64±0,05 μm, а најкраћи су дужине до 0,2 μm. Број микроехина по јединици површине 5 μm x 5 μm износи 17,4±2,4.

КЉУЧНЕ РЕЧИ: морфологија полена, *Campanula lingulata*