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PALYNOMORPHOLOGICAL STUDY OF PRIMROSE (*PRIMULA VULGARIS* HUDS.) GROWN IN NATURAL RESERVE OBEDSKA BARA (SERBIA)

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Abstract: The pollen morphology of primrose (*Primula vulgaris*, fam. *Primulaceae*) has been investigated using light and scanning electron microscopy to contribute to melissopalynological studies of honeys originating from the native apiflora. Palynomorphological investigation included the examination of pollen symmetry, polarity, ornamentation, aperturation, shape and size. The pollen grains are isopolar, radially symmetric and shed as monads. The exine ornamentation is reticulate. Analysis of pollen morphometric characteristics revealed that grains are small to medium size and prolate in shape. Given the aperturation, the number of colpi was mostly variable among individuals with a different type of flower ranging from 6 to 9.

Key words: *Primula*, pollen morphology, pin, thrum, SEM.

Introduction

Primula L., as one of the largest genera of the *Primulaceae* including about 400 species of perennials, is centred mostly in temperate and mountainous regions of the northern hemisphere. *Primula vulgaris* (common primrose), native to Southern Europe, appears in late winter and at the beginning of spring (II-IV) on pasture lands and into the undergrowth. In Serbia, a common primrose is distributed in the deciduous forests, meadows and near the streams, providing early spring bee pasture (Umeljić, 2003). Insect pollinators visit the flowers in search of nectar, which is located at the bottom of the corolla tube. The primrose provides an important early nectar source for bees and other flower visitors.

The flowers of *Primula vulgaris* are known by distyly, which is characterised by the development of long-styled pin flowers with anthers midway down the corolla tube and short-styled thrum flowers with anthers positioned at the mouth of the corolla-tube (Webb and Lloyd, 1986). Heterostyly is common for *Primula*

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genus in which more than ninety per cent of species are distylous (Richards, 1993). Although the pollen morphology in *Primulaceae* has previously been investigated (Wendelbo, 1961; Spanowsky, 1962; Carrion et al. 1993; Anderberg and El-Ghazaly, 2000), no surveys have been found in the literature related to this species exhaustively including a detailed description of pollen sculpturing and correlations between the type of flower and variable number of furrows.

The morphological characteristics of pollen grains of the native population of *P. vulgaris* from Serbia were investigated for better understanding of its reproduction biology, and determination of botanical and geographical origin of pollen within melissopalynological study of honeys obtained from different regions of Serbia.

Material and Methods

Pollen morphology of *Primula vulgaris*, a species of primrose native to Serbia, was examined using light microscopy (LM) and scanning electron microscopy (SEM). The samples were directly collected from sites in the vicinity of Obedska Bara, the Special Natural Reserve located along the Sava River in Southern Srem (Serbia), known as one of the oldest legally protected natural assets in the world. The measurements were based on at least 400 grains from several flowers. For light microscopy, fully matured anthers were removed from the fresh flowers. Pollen was placed directly on slides and observed without mounting media, or prepared according to the standard acetolysis method (Erdtman, 1960) and mounted in glycerine jelly. Observations and measurements were made using a Leica DMSL microscope and IM 1000 software. For SEM studies, the pollen grains were directly placed on prepared stubs, covered with gold (in BAL-TEC SCD 005 Sputter Coater, for 100 sec in 30 mA) and observed with JEOL 6390. A morphological analysis consisted of determining the shape and size of pollen grains by measuring the polar axis and equatorial diameter. Pollen symmetry and polarity, as well as exine ornamentation and aperturation were also analysed.

Results and Discussion

Primula vulgaris, which was selected for palynomorphological examination, is characterised by two flower forms with different style length (Figure 1a, b). The long-styled and short-styled flower forms are also distinguished by the pollen morphology, referring to the various pollen grain dimensions, lumina reticulum diameter and variable number of colpi.

The pollen grains are isopolar and radially symmetric. According to Erdtman's classification (1971) they are small to medium, showing difference in size between pin and thrum flowers (Figure 2). The thrum pollen grains are about 50% larger than the pin pollen grains (Table 1; Figure 2e, f), but polar

axis/equatorial diameter ratio is similar between these two types indicating the same shape. The shape of pollen grains in polar view is circular and in equatorial view is prolate with long and narrow colpi that do not meet at the poles. Elongated apertures are arranged around the equator, and considerable variation between individuals with respect to the number of furrows is evident, ranging from 6-9 (Figure 2a, b, c, d). Pin pollen is usually 6 (-7)-zonocolpate, and thrum is 8-9 (rarely 10)-zonocolpate.

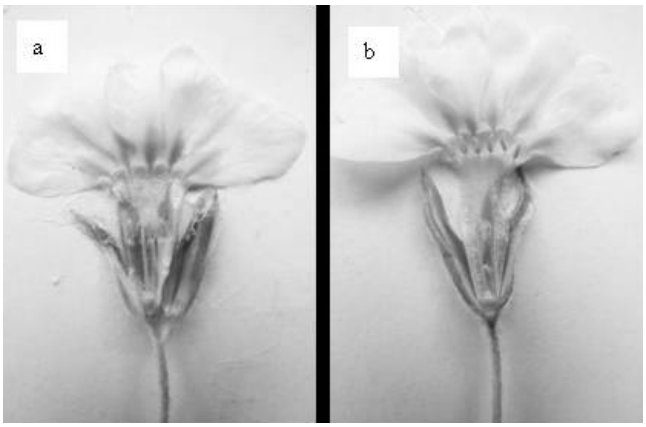

Figure 1. Longitudinal section of distylous flower of *Primula vulgaris* showing stamens inserted unequally: a) thrum-eyed flower having the anthers raised above the stigma near the apex of the corolla tube, b) pin-eyed flower having the anthers half-way down the corolla tube.

Ornamentation is reticulate, and significant differences in lumina size between thrum and pin pollen were recorded. The lumina size was approximately 3 times larger in thrum pollen (Figure 2 g, h), while the muri is similar in both types. Lumina is irregularly polygonal in shape on mesocolpia, with no marked variations in size towards colpate margins, but diminishing in size towards poles forms microreticulate zone.

In the genus *Primula* various types of pollen were registered relating to the shape, number and distribution of apertures: tricoplate, tricolporate, trisyncolpate or polycolpate (Anderberg and El-Ghazaly, 2000). Tricolpate or trisyncolpate pollen types are the prevailing pollen types in this genus. According to Richards (1993) only 11% of total number of species has polycolpate pollen that is known to occur only in a few sections: *Primula*, *Auganthus*, *Pinnatae*, *Armerina*, and *Carolinella* (Anderberg and El-Ghazaly, 2000). Based on our observations, *Primula vulgaris* pollen grains belong to the polycolpate group which is characterised by numerous furrows parallel to the longer axis. The pollen grains of

the *P. vulgaris* are obviously prolate with obtused polar ends, in contrast to pollen grains of 7 species of the genus *Primula* investigated by Anderberg and El-Ghazaly (2000), characterised by prolate-spheroidal shape.

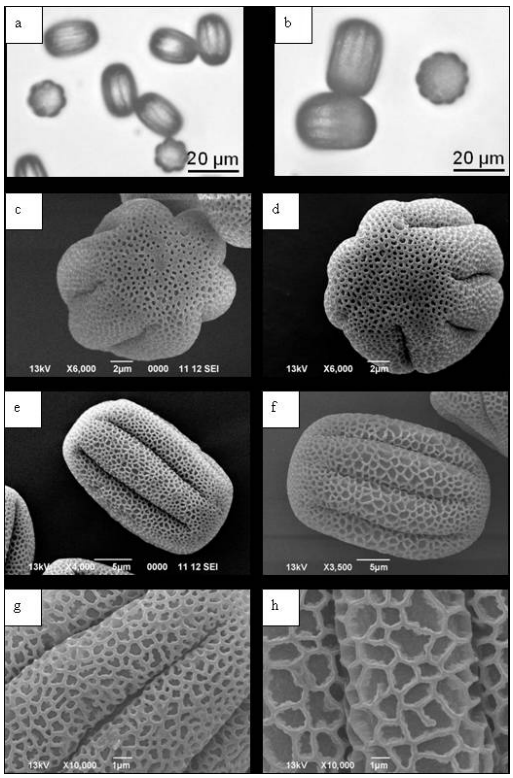

Figure 2. Pin (a, c, e, g) and thrum (b, d, f, h) pollen grains of *Primula vulgaris* comparatively: a-b (LM) – polar and equatorial view, c-d (SEM) – polar view of polycolpate pollen showing 6 and 8 apertures respectively (x6,000), e-f (SEM) – equatorial view (x4,000; x3,500), g-h (SEM) – details of slightly convex mesocolpium ektexine surface showing reticulate ornamentation (x10,000).

Like other species of the genus *Primula* (i.e. *P. veris, P. elatior* and *P. farinosa*) and some other members of the family *Primulaceae*, *P. vulgaris* is heterostylous. The presence of distyly in the *Primula* genus has long been known (Darwin, 1862). Acording to Wedderburn and Richards (1992), more than 90 per cent of the 426 species of *Primula* have distylous populations, as do members of the closely related genera *Hottonia* (one of two species) and *Dionysia* (40 of 41 species). As pointed out elsewhere (Webb and Lloyd, 1986), genetic polymorphism in distylous plants produces two floral types among individuals of a population,

which have anthers and stigma at reciprocal heights. Interesting pollination systems related to dimorphic flowers are specific to the *Primulaceae* family. *Primula* species themselves were subjects of numerous experiments trying to show the advantages of heterostyled flowers (Kurian and Richards, 1997). It is considered that the differences in floral structures function to promote intermorph pollen flow (Dulberger, 1992). Insect pollinators, by inserting its proboscis into a flower, collect pollen that is deposited on the site of the body that is suitable for transferring to the style of the other flower form. In accordance with Barrett (2002), this phenomenon reduces pollen wastage and protects against self-fertilisation and inbreeding depression.

Table 1. Pollen morphometric characteristics of *Primula vulgaris*.

Flower type	P (µm)	E (µm)	P/E	CL (µm)	ML (µm)	L (µm)	M (µm)
pin	19.1 ± 0.5	13.2 ± 1.2	1.5 ± 0.1	14.0 ± 2.3	3.9 ± 0.4	0.5 ± 0.1	0.28 ± 0.03
thrum	31.2 ± 0.9	21.4 ± 1.5	1.5 ± 0.1	23.2 ± 1.3	6.2 ± 0.6	1.4 ± 0.3	0.27 ± 0.05

P-polar axis, E-equatorial diameter, P/E-polar axis/equatorial diameter ratio, CL-colpus length, ML-mesocolpium length, L-lumina, M-muri.

Our study confirmed earlier suggestions that long-styled flowered plants have smaller pollen grains related to short-styled flowered plants and that both types of flowers differ in pollen production, meaning that the first ones produce about two times more pollen grains per flower (Piper and Charlesworth, 1986; Shou, 2008). Richards (1993) supposed that different pollen sizes restrict the possibilities of thrum x thrum and pin x pin crosses, because the pin pollen with lower amount of food reserves is not equipped for sending its germ tube down the length of a pin style and larger thrum pollen is unable to penetrate the surface of a thrum stigma. In favour of cross-pollination is the fact that the stigma of one flower form has short papillae adapted to accept smaller pollen grains from other flower form that has long papillae suitable for larger thrum pollen grains. Even if self-pollination does occur especially in the short-styled flowers, it rarely leads to fertilisation so that heteromorphy in these plants appears to be associated with self-incompatibility (Ornduff, 1980). Earlier studies confirmed that heteromorphy, related to the style length, anther position, stigma shape, stigma papilla length and viability in production of pollen, is genetically controlled and encourages cross-pollination (Wedderburn and Richards, 1992; Al Wadi and Richards, 1993). Kurian and Richards (1997) found that thrum-linked features are genetically dominant to pin-linked features, so that thrums are heterozygotes and pins are homozygotes. Moreover, the gene loci that determine the viability and (sub)lethal properties of short and long homostyle primrose were investigated by Crosby (1949) and Richards (1993).

Although many insect flower visitors other than bees have been observed visiting *Primula vulgaris* flowers, it is not well known if they serve as actual pollinators (Woodell, 1960). It is considered that *Lysimachia vulgaris* L. is the only species in *Primulaceae* significant for bees, visited by the *Macropis fulvipes* F. and *M. labiata* F. (*Melittidae*), and genus *Primula* is sometimes visited by *Anthophoridae*, bumblebees and *Xylocopa* (Simpson and Neff, 1983; Ricciardelli D'Albore and Intoppa, 2000). It is also considered that primroses can only be successfully pollinated by long-tongued bees whose tongues pick up pollen at the right level to transport it to the stigma of the opposite type of plant. In this study, however, pollinator observation indicated that the honey bee was the only flower visitor of primrose, searching for pollen and nectar (Figure 3).

Figure 3. *Apis mellifera* visiting flowers of *Primula vulgaris*, Obedska Bara, Serbia, March 2007.

According to Umeljić (2003), because of the narrow and long corolla tube, honey bees can collect nectar only when the weather conditions are favourable for the intense secretion. Nevertheless, the primrose is considered to be a significant source of pollen, especially in the period of early spring bee pasture, when other plants have not yet begun to flower.

Conclusion

Analysis of pollen morphometric characteristics of primrose revealed that grains are isopolar, radially symmetric, small to medium-sized and prolate in shape. The exine ornamentation is reticulate. Results of palynomorphological investigation indicated that two different forms of flowers produced on separate plants have pollen grains of different sizes. It was established that pollen grains were 6 (-7) or 8 (-9)-zonocolpate and variability in the number of furrows among individuals with a long-styled and short-styled flowered plants was evident.

Acknowledgements

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PALINOMORFOLOŠKO PROUČAVANJE JAGORČEVINE (*PRIMULA VULGARIS* HUDS.) SA PODRUČJA NACIONALNOG REZERVATA OBEDSKA BARA (SRBIJA)

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R e z i m e

Palinomorfološka istraživanja jagorčevine (*Primula vulgaris*) koja su obuhvatila analizu osnovnih karakteristika polenovih zrna kao što su simetrija, polarnost, ornamentacija, aperturacija, oblik i veličina, obavljena su uz pomoć svetlosne i skenirajuće elektronske mikroskopije u cilju doprinosa melisopalinološkim istraživanjima meda poreklom iz apiflore različitih područja Srbije. Polenova zrna jagorčevine su izopolarna i radijalno simetrična, retikulatne ornamentacije. U pogledu aperturacije, evidentna je varijabilnost u broju brazdi na polenovim zrnima sakupljenim sa cvetova različitih individua, te su ona 6(-7) ili 8(-9)-zonokolpatna. Analiza morfometrijskih karakteristika polena pokazala je da polenova zrna po veličini spadaju u grupu malih do srednjih i da imaju prolatni oblik.

Ključne reči: *Primula,* morfologija polena, pin, trum, SEM.

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