

Pollen morphology and flower visitors of *Leiotulus aureus* (Sm.) Pimenov & Ostr. (Apiaceae)

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Abstract – The pollen grains of *Leiotulus aureus* (syn. *Malabaila aurea* (Sm.) Boiss.) were examined by light and scanning electron microscopy in order to contribute to the taxonomical and melissopalynological studies of the species. Flower visitors have also been observed and analyzed aiming at the clarification of some pollination aspects including the species contribution to bee pasture. The pollen grains of *L. aureus* are isopolar, radially symmetrical, medium to large in size, tricolporate and perprolate. They are slightly equatorially constricted with obtuse polar caps and triangular in polar view. The sculpturing pattern is rugulate–microperforate. With regard to flower visitors, the following pollination types occurred: melittophily, myophily and sapromyophily and cantharophily. Some insects attracted by *L. aureus* cannot be considered pollinators but casual visitors. The flowers were the most frequently visited by honey bees during midday.

Keywords: *Malabaila aurea*, light microscopy – LM, palynomorphology, scanning electron microscopy – SEM, insect pollinators

Introduction

According to the latest taxonomic revision, genus *Leiotulus* Ehrenb., belonging to the Apiaceae tribe *Tordylieae* Koch., contains 10 species and subspecies, previously mainly assigned to the genera: *Malabaila* and *Pastinaca* (POWO 2022). Species *Leiotulus aureus* is known under the following homotypic synonyms: *Heracleum aureum* Sm. (basionym), *Lophotaenia aurea* (Sm.) Griseb., *Malabaila aurea* (Sm.) Boiss., and *Pastinaca aurea* (Sm.) Calest., while the accepted name is proposed as a new name combination by Pimenov and Ostroumova (1994), based on carpological investigations. The genus included species characterized as intermediate between *Pastinaca* L. (incl. *Malabaila* s.str.) and *Zosima* Hoffm. (e.g. *Malabaila involucrata* Boiss. & Spruner, *M. pastinacifolia* Boiss. & Balansa etc.).

L. aureus is a herbaceous biennial, a plant of the native range from SE Europe to NW Turkey (POWO 2022), preferring warm and sunny places along with open rocky habitats. It is characterised by a semirosette growth form, erect, hollow, striate and somewhat viscid stem up to 50 cm tall, pinnately divided leaves with ovate leaflets near the ground and linear-lanceolate in upper stem ones. Bright yellow flowers are grouped in terminal and subterminal, regularly compound umbels (Tutin et al. 1981). The mature fruit is obcordate – suborbicular, flattened, surrounded by a wide some-

what thickened margin, cordate at the apex, with persistent style (Fig. 1). Although the plant is slightly aromatic, a literature search did not reveal any reference to its local use in traditional medicine or cooking.

Investigations regarding apiacean pollen morphology have been reported by a number of authors, including Erdtman (1971), Van Zeist and Bottema (1977) and Cerceau-Larrival (1981). However, although pollination biology within the Apiaceae has not received much attention, some aspects were studied in a few taxa including *Chaerophyllum*, *Heracleum*, *Seseli*, *Thaspium*, *Zizia* and *Daucus* (Lindsey 1984, Lindsey and Bell 1985, Pimenov and Ostroumova, 1994, Lamborn and Ollerton 2000, Langenberger and Davis 2002a,b, Rovira et al. 2002, Wróblewska 1993, Mačukanović-Jocić et al. 2016). Besides phylogenetic studies (Pimenov and Ostroumova 1994, Ajani et al. 2008, Downie et al. 2010), scientific papers relating to other reproductive aspects of the *Leiotulus* species are rather rare. Although some palynological studies within the genus *Leiotulus* are modest, except on *L. secacul* (Mill.) Pimenov & Ostr., *L. kotschyi* (Boiss.) Pimenov & Ostr. (Van Zeist and Bottema 1977), the pollen features of *L. aureus* have been unexplored.

The present study aimed to provide palynomorphological features of the species that can be used for the taxon identification and clarification of higher level relationships within the family, as well as to contribute to melissopalyno-

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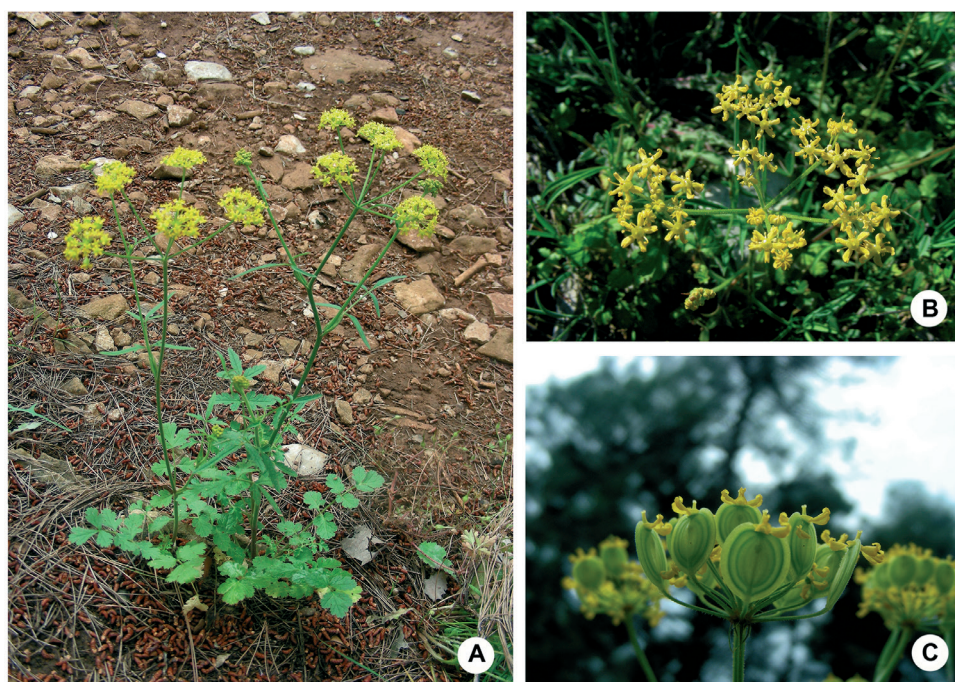


Fig. 1. Habitus of *Leiotulus aureus* (A), inflorescence close up (B), detail of immature fruits (C) (photo: Danijela Stešević).

logical studies and to the pollen atlas of the region. In addition, the study aimed to examine the attractiveness of this species to the honey bee, in terms of its contribution to honey bee pasture, by providing evidence about flower visitors.

Materials and methods

Study site

The research was focused on *L. aureus* plant population in the region of Gorica hill (Podgorica), Montenegro (N 42° 26' 57'' E 19° 16' 2'', elevation 114 m). The population inhabits xerophilous rocky pastures dominated by *Salvia officinalis* L. and *Stipa eriocaulis* Borbás, classified within NATURA 2000 as habitat type 62A0 – Eastern sub-mediterranean dry grasslands (*Scorzoneratalia villosae*). Using D.A.F.O.R. scale for species abundance presence (%): **D** = Dominant (51–100%), **A** = Abundant (31–50%), **F** = Frequent (16–30%), **O** = Occasional (6–15%), **R** = Rare (1–5%), *L. aureus* is observed as “Abundant” in a given area. Pollen collection and insect observations were conducted in 2017 during the blooming period in May. Three mounted and labeled plant voucher specimens (1500402, 1500403, 1500404) were processed and deposited in the herbarium collection of the Faculty of Natural Sciences and Mathematics, University of Montenegro (TGU). Digital photographs of each completed specimen and the accompanying data have also been provided.

Sampling and analysis of pollen

For scanning electron microscopy (SEM) and light microscopy (LM) analysis, the umbels (flowers) at full flowering stage were collected from 10 plants of wild populations.

For SEM study, the pollen grains from fully open flowers were mounted directly on the stub. Aiming to avoid any

deformation of the pollen grains or any swelling attributed to solvents, preparation was carried out without the previous acetolysis method (Dustmann and von Der Ohe 1993). Samples were coated with gold (in BAL-TEC SCD 005 Sputter Coater, 100 seconds in 30 mA) and observed using a JEOL JSM – 6390 LV electron microscope at an acceleration voltage of 20 kV. Pollen grains were photographed in polar and equatorial view, and measurements were done on a sample of 50 or more grains for each morphological character. The following features describing pollen grains were examined: size, shape, ornamentation, apertures, polarity, symmetry, length of polar (P) and equatorial axis (E) (in SEM) and exine thickness (in LM). Description of pollen morphology was performed according to Punt et al. (2007) and Erdtman (1971).

For LM, the pollen grains were mounted on slides in a drop of saturated solution of fructose in water, observed without additional staining with a Leica DM2000 microscope equipped with a digital camera (Leica DFC320) and Leica IM1000 software.

Field monitoring and identification of insect visitors

Field observations were carried out during the peak flowering period of *L. aureus*, from 7 a.m. to 7 p.m., due to the absence of insect activity outside during the rest of the day. Furthermore, nocturnal insects or those active in the evening (such as sphingid moths) were not recorded. In addition to recording honey bee visits, imaging and sampling of all other flower visitors were performed. The insects were photographed with a Nikon Colorpix P500 digital camera. For the purpose of insect sampling, some insects were identified on sight, but for most species an accurate identification was made only after a specimen had been captured.

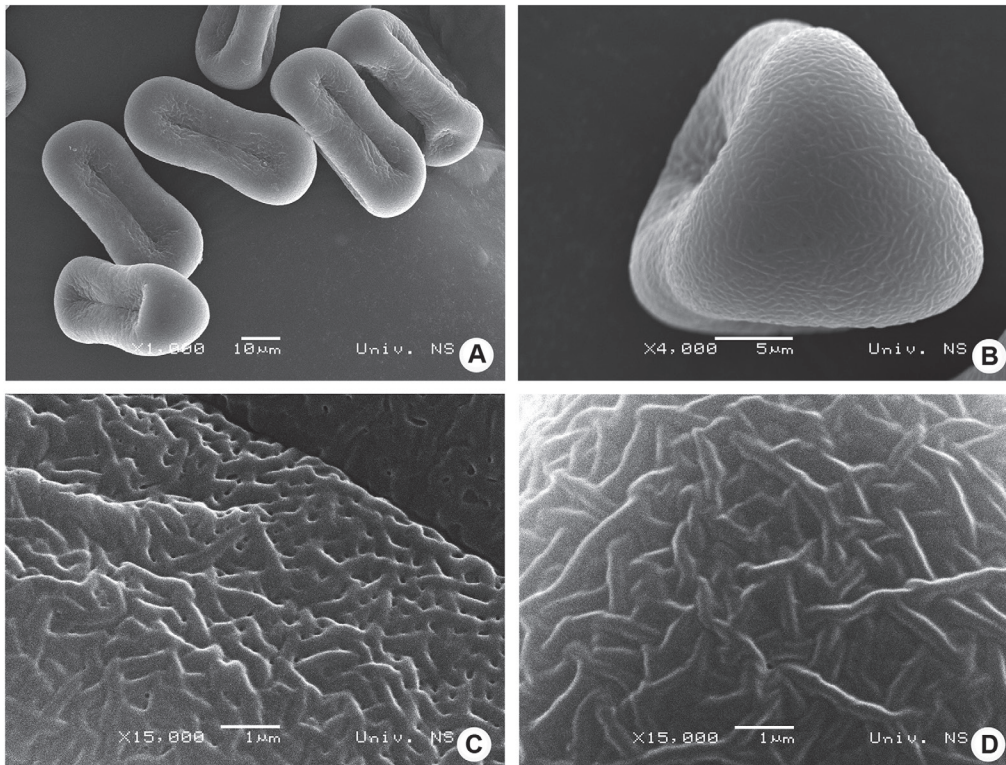


Fig. 2. Scanning electron microscopy of *Leiotulus aureus* (*Malabaila*-type) pollen: the perprolate tricolporate pollen grains are bone-shaped in equatorial (A) and triangular in polar view (B). A detail of exine surface showing rugulate – microperforate ornamentation (C, D).

Specimens were collected by entomological net and exhauster and stored for further determination in the laboratory by experts according to the relevant literature (Bouchard et al. 2011, Cassis and Schuh 2012, Lupoli 2017, Oosterbroek 2006, Sivell 2021, Van Veen 2004, Vazquez 2002).

In order to estimate the frequency of honey bee visits, three plants were randomly chosen and marked at the locality. For diurnal dynamics of frequency of visits, honey bees were monitored five times a day at three-hour intervals. Percentage of visited flowers per plant was calculated as total number of honey bee visits to each marked plant multiplied by the number of umbels visited by one honey bee, divided by the total number of open umbels per plant. The ratio of visited umbels per plant were calculated according to modified formula by Dafni et al. (1988). The following parameters

were monitored in the field: A: The total number of bees visiting per marked plant in a time interval of 5 min, B: The number of umbels that each bee visited on the marked plant, C: The total number of umbels per plant. From these data, the percentage of umbels visited by honey bees was calculated according to the formula as follows: $(A \times B / C) \times 100$. Also, the time each bee spent on the plant was measured.

Results

Pollen morphology

The pollen grains of *L. aureus* are isopolar, radially symmetrical and at the interface between medium-sized and large, according to Punt et al. (2007). The ratio between the

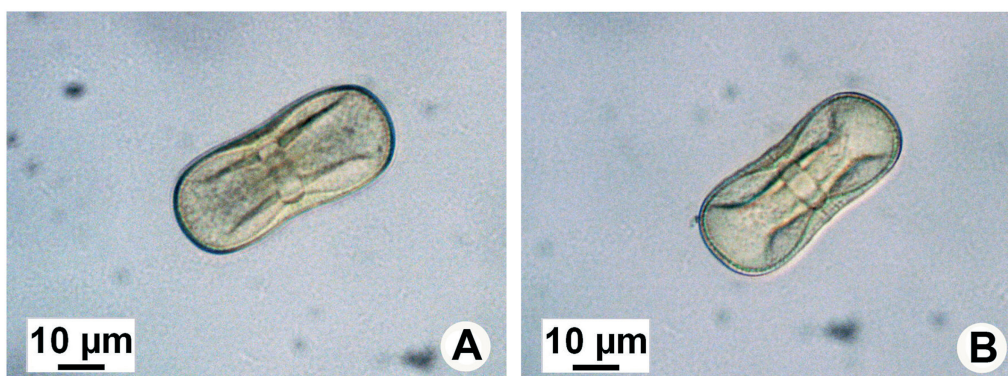


Fig. 3. Light microscopy micrographs of mesocolpial side of *Leiotulus aureus* (*Malabaila*-type) pollen grains showing clearly visible pores (A) and thicker exine (B) in the area of equatorial constriction.

polar axis length ($P = 49.4 \pm 2.1 \mu\text{m}$) and the equatorial diameter ($E = 19.5 \pm 1.1 \mu\text{m}$) amounts to 2.5 ± 0.2 indicating prolate shape. The grains are slightly equatorially constricted with blunt polar ends (Figs. 2A, 3A, 2B), and in polar view they are triangular with interangular furrows (Fig. 2B). The grains are tricolporate with three straight sunken ectocolpi arranged regularly meridionally, of mean length $28.5 \pm 2.2 \mu\text{m}$, each with one endopore positioned in the indentations between the mesocolpial lobes (Figs. 2A, 3A, 2B). Mesocolpial width averaged $9.9 \pm 1.6 \mu\text{m}$. The sculpturing pattern, clearly visible in SEM is rugulate - microperforate (Fig. 2C). Exine is found to be, as observed in LM, $1.22 \pm 0.25 \mu\text{m}$ thick at the poles and twice as thick in the equatorial region ($2.59 \pm 0.49 \mu\text{m}$) (Fig. 3B).

Flower visitors

The golden yellow flowers of this species, arranged in terminally compound umbels, were visited by varyingly efficient pollinator insects. The results of the current study suggest that some floral features are attractive for different insect visitors belonging to four orders: Hymenoptera (*Apis mellifera* – Fig. 4A), Diptera (*Episyrphus balteatus* – Fig. 4B, *Scaeva pyrastris* – Fig. 4C, *Sphaerophoria scripta* – Fig. 4D, *Lucilia* sp. – Fig. 4E, *Sarcophaga* sp. – Fig. 4F, and *Bibio* sp. – Fig. 4G), Coleoptera (*Oedemera lurida* – Fig. 4H, *Mordella aculeata* – Fig. 4I, *Malachius bipustulatus* – Fig. 4J) and Hemiptera (*Closterotomus* sp. – Fig. 4K, *Graphosoma lineatum* – Fig. 4L). These insects are classified as primary and secondary pollinators and or accidental visitors.

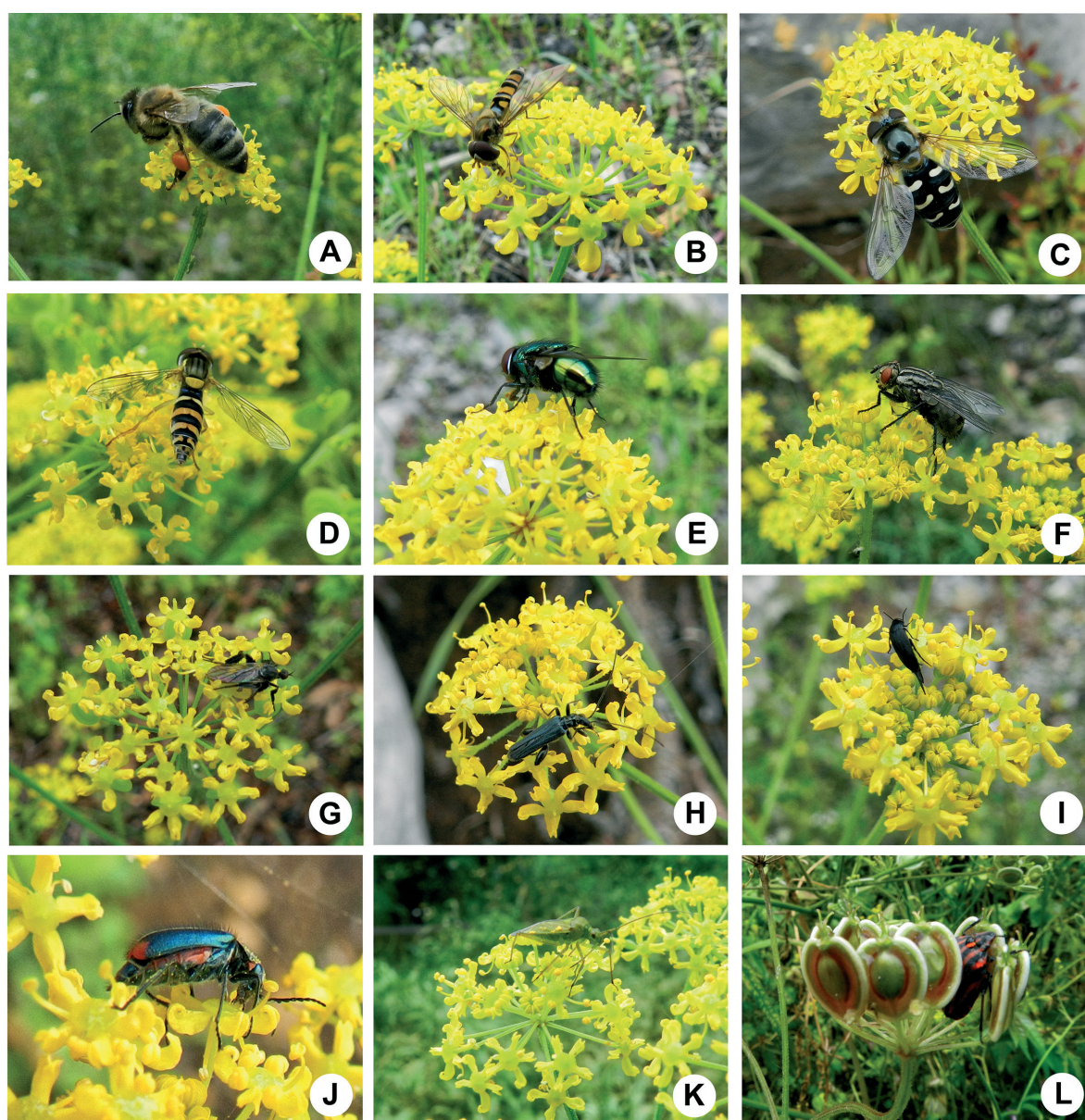


Fig. 4. The flower visitors of *Leiotulus aureus* during the observation period: fam. Apidae (*Apis mellifera* – A), fam. Syrphidae (*Episyrphus balteatus* – B, *Scaeva pyrastris* – C, *Sphaerophoria scripta* – D), fam. Calliphoridae (*Lucilia* sp. – E), fam. Sarcophagidae (*Sarcophaga* sp. – F), fam. Bibionidae (*Bibio* sp. – G), fam. Oedemeridae (*Oedemera lurida* – H), fam. Mordellidae (*Mordella aculeata* – I), fam. Melyridae (*Malachius bipustulatus* – J), fam. Miridae (*Closterotomus* sp. – K), fam. Pentatomidae (*Graphosoma lineatum* – L) among maturing fruits.

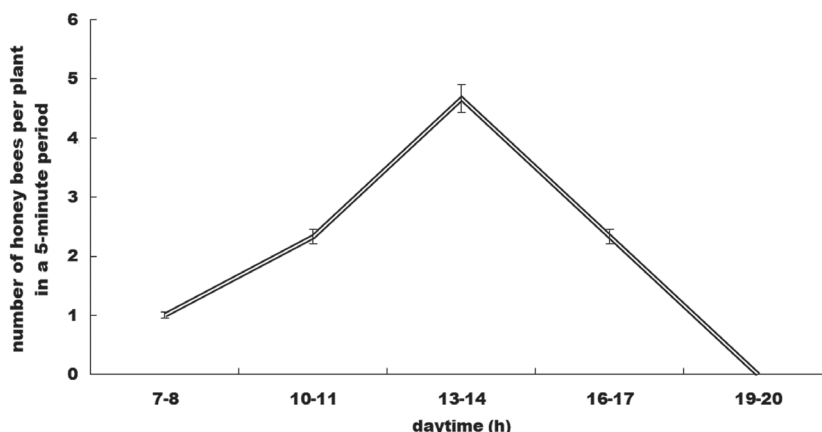


Fig. 5. Diurnal dynamics of honey bee visit frequency to *Leiotulus aureus*.

Regarding the observed flower visitors, pollination types are as follows: melittophily including pollination by honey bees, myophily and sapromyophily including specialized and non-specialized flies, and cantharophily (beetle pollination). Nevertheless, field observations have shown that the plant could be considered melliferous, as the umbels were gladly and frequently visited by honey bees.

During daytime, the average number of honey bees that visited each plant within a 5 minute interval was 2.0 ± 1.7 . From 7 a.m. the frequency of honey bee visits increased reaching a maximum between 1–2 p.m., attaining 4.7 ± 1.5 bees per plant, followed by a decreasing tendency toward the evening and no visitors were recorded during the final observing period (7–8 p.m.) (Fig. 5). The average time a bee spent on the inflorescence amounted 6.06 ± 2.64 seconds (ranging from 3.57 ± 0.60 seconds at 7–8 a.m., to 9.47 ± 1.15 seconds at 1–2 p.m.).

The total number of umbels per visited plant averaged 6.3 ± 0.5 and average flower number per umbel amounted 34.4 ± 5.7 . The percentage of visited umbels per plant during daytime had a pattern similar to that shown by the diurnal dynamics of honey-bee visiting frequency (Fig. 6), reaching a peak between 1 and 2 p.m., with decreasing tendency towards evening.

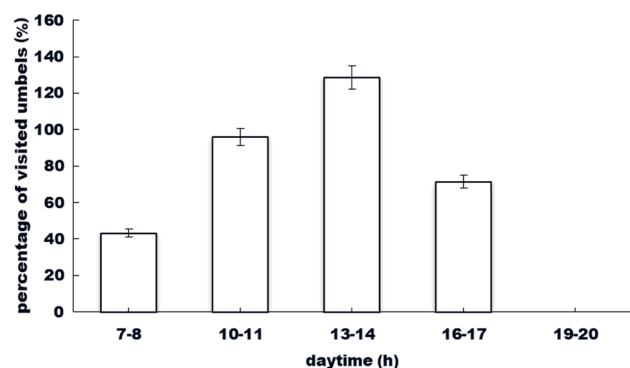


Fig. 6. Percentage of visited umbels per plant during daytime (refers to honey bees). In some periods of the day these values exceed 100%, since it can happen that the same bee during foraging will visit some umbels more than once.

Discussion

Pollen grains within the Apiaceae are usually stenopalynous, radially symmetrical, isopolar and prolate to perprolate in shape (Erdtman 1971). Grains are commonly tricolporate with slit-like ectocolpi and very distinctive and broadband-like costae (Punt 1984, Perveen and Qaiser 2006). The tectum is commonly striate-rugulate or simple striate (Punt 1984), psilate to granulate (Perveen and Qaiser 2006). There are a few different criteria accepted for classification of the pollen grains of the Apiaceae. Various characters have been used, such as polar/equatorial ratio (P/E), exine pattern, etc. For example, Cerceau-Larrival (1962) proposed 5 pollen types occurring within the family based on shape index: sub-rhomboidal (type 1, P/E:1-1.5), sub-circular (type 2, P/E:1-1.5), oval (type 3, P/E:1.5-2), sub-rectangular (type 4, P/E:2), and equatorially constricted (type 5, P/E:over 2). According to this classification, pollen grains of *L. aureus* in the current study should fit into type 5. In addition, according to Van Zeist and Bottema (1977) the pollen grains of the Apiaceae could be divided into 9 pollen types: *Anisosciadium*, *Bunium*, *Bupleurum*, *Eryngium*, *Ferula*, *Malabaila*, *Pimpinella*, *Sium erectum* and *Turgenia* types. The pollen features characterizing the *Malabaila* type are as follows: subrectangular to slightly oval shape with rounded poles, P/E is 2, fairly long colpi, transversal furrow oval-rectangular, distinct columellae 0.5 to 0.8 μ m in diameter, slight variations in the wall thickness (exine up to 2 μ m thick), grain size ranging from 35 to 40 μ m. This type comprises *Malabaila secacul*, *M. kotschy*, *Orlaya grandiflora*, *Heracleum maximum*, *H. lasiopetalum*, *H. persicum*, *Ormosciadium aucheri*, *Scandix iberica*, *Stenotaenia nudicaulis* and *Turgeniopsis foeniculacea*. Recently, *Orlaya* has already been described as having its own pollen type (Beug 2015), while *Malabaila secacul* and *M. kotschy* were transferred to genus *Leiotulus* (as *L. secacul* (Mill.) Pimenov & Ostr and *L. kotschy* (Boiss.) Pimenov & Ostr.) (Pimenov and Ostroumova 1994, POWO 2022). The results of palynomorphological research of *L. aureus* (which previously also belonged to *Malabaila* genus) are not completely in accordance with characters described for the

Malabaila pollen type, such as P/E ratio and grain size, provided by Van Zeist and Bottema (1977). The pollen grains, described in the current paper, are slightly larger. It is known that the size may vary depending on the liquid medium used to mount samples on microslides for light microscopy (Faegri and Iversen 1989, Pospiech et al. 2021). However, the measurements of pollen size in the present paper are based on micrographs obtained by SEM, without using any mounting media which may cause enlargement of pollen grains. Extensive palynological research provided by Punt (1984), who recognized 50 types of pollen, as well as by Perveen and Qaiser (2006) who distinguished three pollen types based on tectum features within 27 Apiaceae genera, did not cover *Malabaila/Leiotulus* species.

The Apiaceae species have a uniform umbel and flower structure, however within the family diverse pollination systems occur varying from completely self-pollination to obligate cross-pollination (Koul et al. 1993). Flowers of umbellifers attract numerous insect taxa from several taxonomic orders supplying them with pollen and nectar (Niemirski and Zych 2011). Hence, umbellifers are often connected with generalized pollination system, indicating that their floral nectar is easily accessible to flower-visiting insects of different pollination efficiency due to the various degree of mouthpart modification for feeding on nectar, pollen or petals (Proctor et al. 1996, Olesen et al. 2007). These include mostly flies, but also hymenopterans and coleopterans (Proctor et al. 1996, Lamborn and Ollerton 2000, Zych 2007, Davila and Wardle 2008, Carvalheiro et al. 2008, Mačukanović-Jocić et al. 2016). In the current study, flowers of *L. aureus*, representing an open dish-shaped blossom type, were visited by twelve insect species from four taxonomic orders. With regard to the observed flower visitors, the following pollination types occurred: melittophily, myophily, sapromyophily and cantharophily. Dipterans were the most frequent group of visitors. Most of the recorded dipterans were of the “hoverfly type” (i.e. members of the family Syrphidae) and the “muscid type” (i.e. members of the families Sarcophagidae (flesh flies) and Calliphoridae (blow flies)). Although not highly specialized in pollination, flower-pollinating species have been reported in the Bibionidae in previous research (Goldblatt et al. 2005). Adults of march flies (Bibionidae) are known as pollen-collecting and nectar feeding flower visitors pollinating fruit trees and some other crops (Freeman and Lane 1985, Woodcock et al. 2014). In the present study carrion flies were found to be less important from the pollination point of view, since *Leiotulus* is an aromatic plant more attractive to Apidae and Syrphidae. Despite a few Coleopteran species observed in the current and similar studies on umbellifers (Zych 2006), they are of minor importance as they are not considered to be efficient pollinators (Mačukanović-Jocić 2010). Hemipterans observed in this study could not be considered as pollinators, since their pollination activity is negligible, except in rare cases (Ishida et al. 2009). Unlike previous research on some other Apiaceae species (Zych 2006, Mačukanović-Jocić et al. 2016), in the present study neither moths nor butterflies

were observed visiting *L. aureus* umbels. Low visitation rate of lepidopterans can be explained by the length of their proboscis, which is more adapted to tubular corollas, or by weaker attractiveness of flowers which can be attributed to the scent or floral nectar components. *L. aureus* could be considered an ecologically generalized species in terms of the need for specialized pollinators. There are many reasons for this. Although *L. aureus* like many other umbellifers lack any visible or invisible floral signs such as honey guides leading to nectar, the flowers are slightly aromatic and could emit some chemical signals that attract specific groups of pollinators, as previously reported for this family (Tollsten et al. 1994, Tollsten and Øvstedal 1994, Niemirski and Zych 2011). Regardless of their floral uniformity, some umbellifers are suggested to exhibit cryptic flower specialization enabling oligolectic relationships with bee pollinators (Lindsey 1984, Lindsey and Bell 1985, Niemirski and Zych 2011). Unlike Zych (2006) who did not observe any honey bee on *Heracleum sphondylium*, *L. aureus* flowers were very frequently visited, which is in line with the findings of other authors who pointed out the importance of honey bees in pollinating umbellifers (Langenberger and Davis 2002b, Davila and Wardle 2002). Following the diurnal dynamics of honey bee visits, plants were the most frequently visited about midday. Considering the frequency of honey bee visits *L. aureus* could be regarded as a melliferous plant contributing to honey bee pasture. However, regarding its rather “unspecialized” floral morphology and its being visited by numerous species of flower visitors, this plant species is without ecological specialization to particular insect species.

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