**Acta Entomologica Serbica, 2021, 26(2): 37-63**

UDC: 595.794/.799(497.11+497.6)
DOI: 10.5281/zenodo.5537059

# FURTHER RANGE EXPANSION OF THE SCULPTURED RESIN BEE (*MEGACHILE SCULPTURALIS*) IN SERBIA AND BOSNIA & HERZEGOVINA

JOVANA BILA DUBAIĆ[1]*, JOVANA RAIČEVIĆ[1], MILAN PLEĆAŠ[1], JULIA LANNER[2], PETAR NIKOLIĆ[3], VLADIMIR ŽIKIĆ[4], LJUBIŠA STANISAVLJEVIĆ[1] and ALEKSANDAR ĆETKOVIĆ[1]

1 Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia
*E-mail: jovanabila@bio.bg.ac.rs (corresponding author)

2 Institute for Integrative Nature Conservation Research,
University of Natural Resources and Life Sciences, Vienna, Austria

3 Faculty of Agriculture, University of Banja Luka, Bulevar vojvode Petra Bojovića 1A,
78000 Banja Luka, Republic of Srpska, Bosnia & Herzegovina

4 Faculty of Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

## Abstract

*Megachile sculpturalis* is the first non-native bee species established in Europe, originating from East Asia. Since early detections in SW Europe (2008–2010), its spreading has resulted in a range currently spanning nearly 2,800 km x 1,100 km across southern and central Europe. In SE Europe, its establishment was confirmed since 2015 in NE Hungary, followed by detection in N Serbia (2017), and with wider spreading across the eastern Pannonian Plain (2018–2019); eventually it was detected in NW Bosnia & Herzegovina (2020). Accordingly, there have been repeated calls for monitoring the spread of *M. sculpturalis*, to address its potential invasiveness, but a more elaborate assessment protocol is still lacking. A working concept for the comprehensive monitoring was proposed in the survey conducted in Belgrade (Serbia) during 2017–2019, based on a quantitative assessment of bee population trends in relation to focal plant resources. There was a need to improve and broaden this initial framework, e.g., to allow for different spatio-temporal scales and potential usage requirements. Therefore, in 2020 we considerably extended the research scope, defining it at two spatial scales: LOCAL, for the Belgrade area – the continuation of protocol development, through a high-intensity assessment of *M. sculpturalis* abundance, bionomics, and distribution, in parallel with an assessment of an extended set of relevant plants (and potential bee-plant interactions); and REGIONAL – a survey covering bee spread across Serbia and Bosnia & Herzegovina, aiming to provide a reference time section in expanding the SE European front while also extending knowledge of its environmental affinities. The study included the launching of a pioneering citizen science project (CSP), which enabled a remarkable geographic coverage in spite of the limited return of positive reports.

The Belgrade-scale survey yielded a modest increase in recorded locations relative to 2019, but recording efficiency was decreased, despite a much intensified surveying efforts and extended coverage. This corroborated the importance of the interseasonal variation of key food resources, which affects both population dynamics and the detectability of this bee through alternating concentration and dilution effects. We confirmed a strong association of detection success with the availability and variability of blooming *Styphnolobium*, at both scales, indicating the importance of including this plant in monitoring assessment protocols. The established phenological extent of *M. sculpturalis* activity (>70 days) also closely corresponded with the phenology of *Styphnolobium* blooming, yet it does not represent the entire phenological span for the region. Almost no records came from surveying other plants. The regional expansion of *M. sculpturalis* during 2017–2020 is documented from 19 wider locations (16 added in 2020). It is particularly well established in the Pannonian, and to a lesser extent in peri-Pannonian area of Serbia and B&H, while the approximated range extent was likely doubled during 2019–2020. Further south records were scarce, indicating a slower expansion across the hilly-mountainous part of the Balkans. Records largely came from urban or other settlements, with only about a third from semi-natural or agricultural environments.

KEY WORDS: invasive alien bees; introduced pollinators; southeastern Europe; monitoring; citizen science

## Introduction

Sculptured resin bee (Hymenoptera: Anthophila: Megachilidae: *Megachile sculpturalis* Smith, 1853) is one of the very few non-native bee species in Europe (Russo, 2016; Rasmont *et al*., 2017; Bortolotti *et al*., 2018). It was the first to be established, and so far, the only one widely distributed across the continent and continuously spreading at remarkable rates (Bila Dubaić & Lanner, 2021; Le Féon *et al*., 2021). This solitary bee typically nests in pre-existing cavities in dead wood, various hollow plant stems (e.g., large reed internodes), but also in diverse man-made structures and materials, with an univoltine life cycle (Maeta *et al.,* 2008; Quaranta *et al.,* 2014; Aguado *et al*., 2018; Ivanov & Fateryga, 2019). The mode of aboveground cavity nesting likely facilitated its accidental introductions, both overseas and within newly colonized continents, through the inadvertent transport of brood concealed in wood/timber and other suitable goods (Mangum & Brooks, 1997; Quaranta *et al.,* 2014; Westrich *et al*., 2015; Russo, 2016; Le Féon *et al.,* 2018; Lanner *et al*., 2020a). Accordingly, its chances for further passive dispersal (secondary introductions) are related to the topology and frequency of 'vectoring goods' transportation, providing that sufficient local population build-up was attained (Bertelsmeier & Keller, 2018). Regardless of accidental human vectoring, it is expected that *M. sculpturalis* possesses a remarkable capacity to spread actively across newly colonized areas (Quaranta *et al.,* 2014; Westrich *et al*., 2015) within regions with adequate resources and the basic environmental conditions.

Sculptured resin bee is native to eastern Asia, where it is relatively widespread and moderately common in eastern China, Korea and Japan (Batra, 1998; Wu, 2006; Ascher & Pickering, 2020). In the early 1990s, it was first successfully introduced into North America (Mangum & Brooks, 1997), followed by rapid range expansion across the eastern half of the continent (Mangum & Sumner, 2003; Hinojosa-Diaz *et al*., 2005; Parys *et al*., 2015). Its second non-native range establishment took place in southwestern Europe: starting from restricted areas in SW France, NW Italy and S Switzerland (2008/2009/2010), its initially slow continuous expansion remained mostly confined to the wider neighboring regions of France and Italy (Vereecken & Barbier, 2009; Amiet, 2012; Quaranta *et al*., 2014; Westrich *et al*., 2015; Le Féon *et al*., 2018; Ruzzier *et al*., 2020). A more rapid spreading was documented since 2014–2015, resulting in a remarkable range extension: throughout the southern half of France, through northern Switzerland and southern Germany to western Austria, throughout most of Italy, eastwards to Slovenia and southwestwards into NE Spain (Aguado *et al*., 2018; Gogala & Zadravec, 2018; Le Féon *et al*., 2018, 2021; Ortiz-Sánchez *et al*., 2018; Lanner *et al*., 2020; Ruzzier *et al*., 2020; Westrich, 2020). In contrast with this largely continuous, i.e., diffusive mode of spread, several relatively isolated establishments took place, mostly across central and eastern/southeastern Europe during 2015–2020: to NE Hungary (Kovács, 2015), NE Austria (Westrich, 2017), N Serbia (Ćetković & Plećaš, 2017), W&S Croatia (Resl, 2018; 'pitrusque', 2019), the Crimean Peninsula (Ivanov & Fateryga, 2019) and NW Bosnia & Herzegovina (Nikolić, 2020). Some of these cases appear to be genuine long-distance 'jumps' (of uncertain origin – cf.

Lanner *et al*., 2021), others being arguably a combination of both dispersal mechanisms, which is yet to be clarified. The capacity of *M. sculpturalis* for remarkable jump-dispersals was most recently demonstrated (2020) through introduction into the Mallorca Islands, across the western Mediterranean Sea (Ribas Marquès & Díaz Calafat, 2021). On the other hand, a study comparing the early-phase colonization in the Belgrade area (N Serbia) with the early spreading of *M. sculpturalis* across the eastern Pannonian Plain during 2015–2019 (Bila Dubaić *et al*., 2021 [in rev.]) suggested a likely mixed mode: a long-distance jump into NE Hungary, followed by continuous diffusive spreading southwards into Serbia. Comprehensive phase-mapping compilations of the colonization history in Europe (Ćetković *et al*., 2020; Le Féon *et al*., 2021) show that it is currently spanning nearly 2,800 km W–E and more than 1,100 km N–S.

There is growing worldwide concern about the current extent of and trends in alien bee introductions with regard to their potential to become invasive, i.e., to cause various negative environmental impacts (Goulson, 2003; Stout & Morales, 2009; Aizen *et al*., 2014, 2020; Russo, 2016; Morales *et al*., 2017; Vanbergen *et al*., 2018). Unlike many other alien insects, the introduction of bees may represent a controversial subject because of the possible overlap of certain negative impacts (either documented or assumed) and the seemingly positive net contributions to pollination services (Russo, 2016). The very term invasive (also: invasiveness, invading, etc.) in the case of *M. sculpturalis* has often been used inconsistently and/or loosely with respect to the 'conceptual issue of impact' in invasion biology (Bila Dubaić *et al*., 2021 [in rev.]). Nevertheless, it is of prime importance to evaluate whether *M. sculpturalis* could cause significant adverse effects on native bee populations (principally through competition for floral and nesting resources), native and exotic flora, and intricate pollination interactions across diverse ecosystems and habitat types (Russo, 2016; IUCN, 2020; Ribas Marquès & Díaz Calafat, 2021).

*Megachile sculpturalis* excessively visits several widely available mass-blooming plants (Quaranta *et al*., 2014; Parys *et al*., 2015; Le Féon *et al*., 2018; Ruzzier *et al*., 2020), hence, a substantial usage overlap with some common generalist bee taxa is obvious, yet no evidence exists of effective competition (in terms of measurable impacts). As for the interactions at nesting sites, evidence was accumulated across both sections of its non-native range (North America and Europe) about the unusually aggressive and/or destructive habits of *M. sculpturalis*, affecting the adults and/or larvae of native solitary bees (*Xylocopa*, *Osmia*, *Megachile*, *Heriades*) or other co-occurring Hymenoptera (most recent summaries in: Le Féon *et al*., 2018, 2021; Lanner *et al*., 2020a,b; Straffon Díaz *et al*., 2021). Additionally, negative correlation has been found between the abundance of *M. sculpturalis* and the presence of native bees in a study based on bee hotels in an urban setting of SE France (Geslin *et al*., 2020). However, we still lack the exact approach to estimating extended impacts on affected taxa, e.g., through causative effects on population trends. Further concerns are expressed about the risk that *M. sculpturalis* could enhance the propagation of invasive plants (Mangum & Sumner, 2003; Aguado *et al*., 2018); although not yet adequately evaluated, concerns seem particularly justified in the case of some exotic Fabaceae, e.g., genera *Pueraria* and *Lespedeza* (Batra, 1998; Lindgren *et al*., 2013; European Commission, 2020). Therefore, following the precautionary principle, *M. sculpturalis* should be regarded as potentially invasive (i.e., a possibly harmful alien species), regardless of the current lack of decisive proof of measurable impacts (cf. Stout & Morales, 2009).

Sculptured resin bee is often referred to as polylectic, with a remarkably high incidence of visitations to exotic ornamental taxa, but also with very strong preference for the pollen of large-flowered Fabaceae; the ornamental Japanese pagoda tree (*Styphnolobium japonicum* (L.) Schott) is well established as the single most frequently used pollen source in Europe (Mangum & Brooks, 1997; Mangum & Sumner, 2003; Maeta *et al*., 2008; Quaranta *et al*., 2014; Parys *et al*., 2015; Westrich *et al*., 2015; Aguado *et al*., 2018; Le Féon & Geslin, 2018; Le Féon *et al*., 2018; Guariento *et al*., 2019; Ruzzier *et al*., 2020). Arguably, some of the interpretations of the plant usage pattern might prove to be biased and/or even inaccurate, but

*M. sculpturalis* undoubtedly visits a wide range of plants, with varying frequencies in different regional settings (cf. Ćetković *et al*., 2020; and ongoing study). Hence, it is of great practical importance to further clarify and to rank genuine sculptured resin bee preferences and visitation patterns in the context of varying phenology and local availability of different floral resources – i.e., to evaluate them also as potential 'monitoring plots'.

Since its introduction, it has been repeatedly proposed that the monitoring of *M. sculpturalis* spread in Europe needs to be established (Quaranta *et al*., 2014; Aguado *et al*., 2018; Le Féon *et al*., 2018; IUCN, 2020; Ruzzier *et al*., 2020; Ribas Marquès & Díaz Calafat, 2021). The monitoring efforts should aim to provide a timely evaluation of bee invasiveness, and in turn, to inform actions for the timely prevention of possible negative consequences. Current ongoing efforts, however, mostly represent an opportunistic documenting of its spread, through the compiling of new occurrence data from a variety of sources. Hence, we lack more specific protocols for assessing the impacts or other relevant parameters. Based on outcomes from the Belgrade survey 2017–2019, Bila Dubaić *et al*. (2021 [in rev.]) established an explicit spatio-temporal framework for the quantitative assessment of bee population trends in relation to focal plant resources, as a working concept for building a more comprehensive monitoring of *M. sculpturalis*. This initial 2019 framework needs to be rigorously tested and 'calibrated' for different spatio-temporal scales and specific purposes. To enable the broad array of current and future requirements, we are currently working towards the following operative targets: (i) to refine and standardize tailored protocols for quantitative assessments in order to provide comparable population estimates across spatial scales and phases in different colonization timelines, (ii) to extend the protocols to account for different combinations of target plants across regions and environment types (from urban to natural) and in variable phenological regimes, (iii) to outline options for flexible monitoring intensity (i.e. various extent of engagement, research or management interests/priorities, etc.). Furthermore, the assessment approach based on recording bee activities on flowers should be integrated with nesting-based monitoring, which is particularly important to complement the evaluation of its potential invasiveness (cf. experiences from: Geslin *et al*., 2020; Lanner *et al*., 2020a,b; Straffon Díaz *et al*., 2021; see also: MacIvor & Packer, 2015).

Accordingly, in the season of 2020 we considerably extended the research programme on *M. sculpturalis* spread in the area, building on previous surveying experiences (in Serbia), as well as on the advancement of respective Europe-wide research (Le Féon *et al*., 2018, 2021; Lanner *et al*., 2020a, 2021; Ruzzier *et al*., 2020). In this phase, it is still an exploratory endeavor with an open-ended timeline regarding the outlined targets. We defined a two-scale approach: LOCAL, for the Belgrade area – the continuation of protocol development through a high-intensity assessment of sculptured resin bee abundance, bionomics and distribution across the main urbanistic-landscape zones, with parallel assessment of the most relevant plants (distribution, phenology, quantification of floral resources), as well as all aspects of bee-plant interactions; REGIONAL – extension of the survey on the entire distribution across Serbia, aiming to track the bee spreading in 'real-time' and to extend evidence of its wider environmental affinities; the survey coverage was eventually extended to neighboring Bosnia & Herzegovina, following the first detection there in August 2020. Herewith we provide a reference time-section in *M. sculpturalis* expansion across SE Europe by documenting presences/absences and abundance trends (i.e., an updated state of the expansion front). As such, this contribution represents a 'progress report' aimed at providing a timely evidence base for diverse planned or ongoing monitoring efforts.

## Material and Methods

### Belgrade survey: a local scale approach

The Belgrade-level survey included a wide array of activities (conducted by JBD, JR, MP and AĆ): (a) checking for *M. sculpturalis* presence at all locations with suitable plants, taking particular care that all target plants within a single location are surveyed simultaneously (wherever two or more plant taxa were available in proximity); (b) wherever found, the assessment of *M. sculpturalis* activity density; this included testing the improved 2019 protocol (a better defined bee-counting procedure, allowing for the highly variable dynamics of bee activity on site, etc.); (c) an extensive assessment of the 'resource units' of selected plant genera across Belgrade urbanistic zones, aiming to detect and assess as many plant sites as feasible (at known locations or through the search for new ones; followed with efforts in refining protocols for different types of plants).

The city of Belgrade was the core study area for our 2017–2019 survey of *M. sculpturalis* establishment in Serbia (Bila Dubaić *et al*., 2021 [in rev.]); therein we elaborated on its most relevant biogeographical, ecological and urbanistic features (available in extensive form online: https://srbee.bio.bg.ac.rs/english/belgrade-general-features). In particular, we introduced the operative concept of wider 'urbanistic-landscape zones' suited for this taxon-specific study, based on elements generally relevant to wild bee studies in an urban setting. The approach provided a simplified 'summary account' of multiple key factors and resources (of importance for bees) across environmental gradients of a large, heterogeneous and dynamic urban area (https://srbee.bio.bg.ac.rs/english/m-sculpturalis-2019-survey). The studied portion of Belgrade municipalities was principally delimited by the availability of sites with plants of interest.

In 2020, we maintained the same study area and conceptual framework: the principal focus was on *Styphnolobium*, as the key food-plant resource, and we broadened the survey to assessing the suitability and relevance of other attractive plant taxa. In this phase of protocol development, we considered all plants known to be frequently visited (anywhere, but principally based on European evidence; cf. Ćetković *et al*., 2020: an ongoing study) while also being locally available and phenologically suitable for this bee species (and accessible to observers). Generally, the relevance for monitoring should be tested regardless of the mode of usage (whether a plant is foraged for pollen or only for nectar), or plant nativeness (NB: this trait may differ at the continental *vs*. regional or local scales for some genera). For the season of 2020, we selected seven prospective genera, representing different plant families, for suitability-testing: *Buddleja* (Scrophulariaceae), *Catalpa* (Bignoniaceae), *Koelreuteria* (Sapindaceae), *Lavandula* (Lamiaceae), *Ligustrum* (Oleaceae), *Lythrum* (Lythraceae) and *Wisteria* (Fabaceae). Within the Belgrade area (and Serbia generally), most of the selected taxa are exotic (except *Lythrum* and *Ligustrum*), and are mostly available through ornamental planting (except *Lythrum*), principally in public spaces.

For the spatial quantification of *Styphnolobium* resources, we started from the framework based on circular 'landscape sectors' (r=250 m), as defined in the 2019 survey (https://srbee.bio.bg.ac.rs/english/m-sculpturalis-2019-survey). In 2020 we aimed to improve the completeness of spatial coverage and resource quantification (Fig. 1A); in particular, we verified and/or complemented estimates of floral resources from 2019. Furthermore, we performed extensive and meticulous assessment of the phenology pattern of *Styphnolobium* blooming across the study area. For other selected plants, we focused on assessing their phenological suitability and attractiveness, particularly considering the comparative availability of *Styphnolobium* resources nearby. Due to various limitations (see in Results), only the subset of these additional genera was feasible to cover with thorough assessment in 2020 (Fig. 1B).

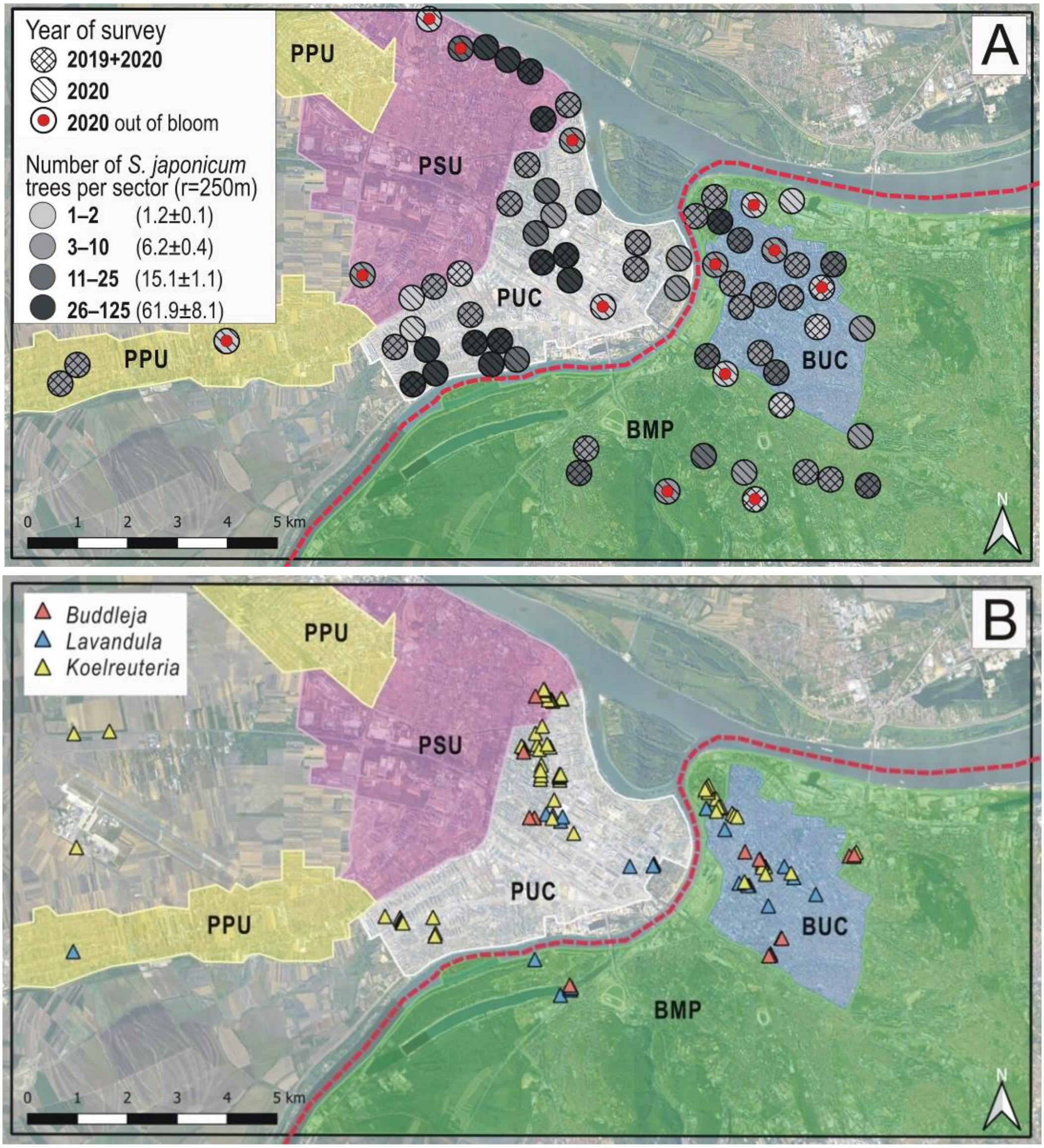


Figure 1. The studied area of Belgrade, showing distribution of principal focal plants included in the 2020 survey. A – Distribution of all detected *Styphnolobium* trees (*S. japonicum*): the main floral resource for *M. sculpturalis* is treated also as most relevant 'unit-plot' for monitoring; numbers of recorded trees are aggregated within r=250 m circular sectors framework (in four abundance classes), in accordance with the approach designed for the 2019 survey (Bila Dubaić *et al.*, 2021 [in rev.]); 13 sectors assessed only outside the blooming period are marked with red dot. B – Distribution of some other focal plants surveyed in 2020, as prospective complementary resources and detection plots (symbols show actual locations of plant units, i.e. without aggregated quantification). The base-map is satellite imagery from Google Satellite™; coloured map overlays represent the customised landscape/urbanistic zonation concept, as elaborated at https://srbee.bio.bg.ac.rs/english/m-sculpturalis-2019-survey: BUC – Balkan Urban Core; BMP – Balkan Mixed Periphery; PUC – Pannonian Urban Core; PSU – Pannonian Semi-Urban; PPU – Pannonian Peri-Urban.

For accurate recording of bees in the crowns of higher trees, it was necessary to use binoculars (Fig. 2B). For each 'unit-point' survey for bee presence, we recorded the time spent in effective personal engagement, typically in focused observations; if both collecting and observation were conducted, effort-time was recorded separately for each activity. A targeted standard duration of observations per 'unit-point' was ≥10 minutes, although shorter (e.g., casual/accidental) observations were also considered eligible for the effort-time estimates. On some occasions where no bee activity could be noted during the 10' period (despite ample blooming of *Styphnolobium*), the observation time was opportunistically extended. The longer time should emphasize the difference between states of very low bee abundance/activity and effective absence (to be interpreted at the relevant landscape scale). According to the previous Belgrade study in 2019, all detections could be interpreted also as timed counts and converted into appropriate abundance estimates (per unit-time). Even within a highly variable and uneven surveying regime, a reference effort-time should enable comparisons both for detection efficiency and for population dynamics parameters.

We conducted surveys of plants in suitable phase from the end of May (30th), until early September (04th), with variable intensity and coverage. It was four weeks before the first detection of *M. sculpturalis* in 2020, and surveys continued for more than two weeks after the last detection in the area (see in Results). Due to the complicated phenology of different plants at the landscape scale, our field work regime was flexibly adjusted throughout the blooming season, varying in intensity/frequency, spatial coverage and floral focus (i.e., optimized within logistical limitations). On a whole-season basis, our field engagement spanned 60 calendar days, with varying per-day personal efforts and per-location time spent; 37 days were within the period of confirmed *M. sculpturalis* activity in the Belgrade area in 2020.

As a trial attempt, in early July 2020 we installed a series of nesting facilities – 'trap-nests' (by LjS, JBD, MP, JR, AĆ) across the wider Belgrade area (16 locations) and in Sremski Karlovci, 60 km to the northeast of Belgrade (where activity of *M. sculpturalis* was confirmed earlier). Nests were prepared as bundles of common reed (*Phragmites*), each with about 16–17 reed internodes of suitable diameter (9–11 mm). Nests were collected in mid-September, after evidencing that *M. sculpturalis* activity ceased all over the area. We preliminary inspected all the reeds and separated those that were inhabited, to be reared in laboratory under the suitable temperature regime (simulating the respective seasonal conditions before the expected period of emergence).

Beyond the Belgrade area: CSP and a regional scale survey

To extend the territorial coverage of *M. sculpturalis* distribution across Serbia, from the season of 2020 we initiated a comprehensive long-term collaborative research (Ćetković *et al*., 2020). This included the launching of a pioneering citizen science project (CSP), an approach that has proved to be highly effective in providing respectable biodiversity data coverage for large areas (Theobald *et al*., 2015; Soroye *et al*., 2018).The launching was largely facilitated through the experience exchange and coordination of activities with the ongoing CSPs established earlier for Alpine countries (Lanner, 2018–2019; www.beeradar.info). We created a thematic web page (https://srbee.bio.bg.ac.rs/azijska-pcela-smolarica/azijska-pcela-projekat-ucesce) with all the relevant information about the sculptured resin bee, our research, and specifically, the CSP approach; we also prepared poster-calls with standardized information (by JBD, JL, AĆ, MP, JR and LjS) that included where, when and how to search for sculptured resin bee, how to recognize it, how to submit a report, etc. Posters were distributed through several social media channels and nature platforms, as well as various other internet sites with relevant focus, while several national and local media took part in dissemination and promotion. We also used all other means of electronic communication to circulate the calls through academic and/or professional networks in Serbia (relevant university departments, scientific societies, beekeepers' associations, etc.), but also through personal contacts of the authors (LjS, JBD, MP, VŽ).

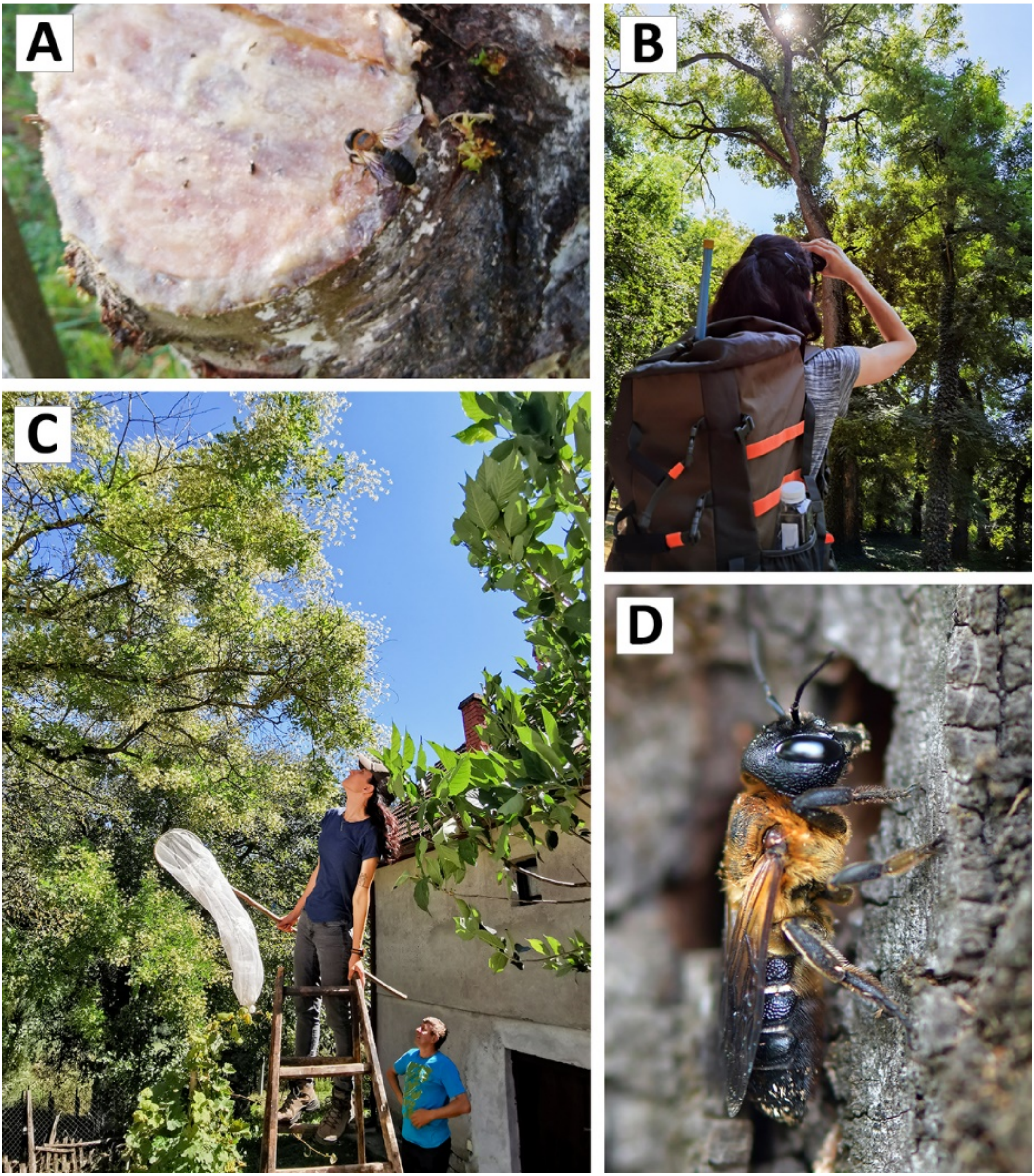


Figure 2. Facets from the 2020 survey: A – *M. sculpturalis* female collecting fresh grafting wax from a cherry tree: Pučile near Bijeljina (CSP report, rec_#44); B – the use of binocular is essential for assessing the presence/activity density of *M. sculpturalis* in a high crown of *Styphnolobium* trees: surveying in Temerin, rec_#34; C – sampling of *M. sculpturalis* on the very late-blooming *Styphnolobium* trees (Sept 05, rec_#64) in a small and remote rural setting of Skržuti, within a semi-natural surrounding: the southernmost record and the 'wildest' of all landscapes we surveyed in 2020 (following the CSP report: rec_#63); D – *M. sculpturalis* nesting in a tree trunk of the semi-withered *Tilia* tree (holes by wood-boring beetles): churchyard in the centre of Ada (CSP report, rec_#42). Photo credits: (A) Nikola Simanić, (B, C) Đorđe Dubaić, (D) Gergely József.

The CSP officially started in early July, after we confirmed the first appearance of *M. sculpturalis* in 2020, and lasted until the end of the summer (https://srbee.bio.bg.ac.rs/azijska-pcela-smolarica/azijska-pcela-projekat-ucesce/gra%C4%91ani-koji-su-dali-svoj-doprinos-na%C5%A1em-projektu). For each report of observed specimen(s), we asked participants to provide relevant accompanying information. Photo- or video-evidence was required, along with the date of observation and detailed location descriptors (if possible, with accurate coordinates). Other details we asked for were not obligatory (site features/circumstances, nesting, foraging on a plant, etc.), but considered as highly valuable and desirable. For each report, we established direct communication with CSP participants to provide feedback, but also to seek additional details (communication mostly by JBD). The verification of specimen identity was based on thorough examinations of the provided photos/videos, and often included repeated communication with participants (coordinated mostly by JBD, double-checked where necessary by AĆ); occasionally, participants also provided collected specimens. Upon verification, we personally visited eight locations of confirmed reports (by JBD and JR) to further explore relevant details of bee occurrences in different environments of Serbia (abundance, host plants or nesting details, habitat/landscape features, etc.). We also used the opportunity to verify the reported location accuracy, and to promote closer communication with citizens for future participation in prospective monitoring networking (but also to sample bee specimens – see more details below).

In addition to visiting the CSP-reported sites, we managed to extend the survey to several other locations in Serbia (by JBD and JR), principally in Vojvodina province (N Serbia): in the period July 05 – Sept 04 we visited 29 sites (within 12 wider settlements), and at 27 of them we located and observed *Styphnolobium* trees (mostly in the blooming phase). A small-scale but important search for *M. sculpturalis* presence was conducted in SE Serbia (by VŽ) within the wider municipality of Niš, as our southernmost-positioned research sites in 2020. The search was conducted by extensive and repeated observations in July – August at two sites with numerous *Styphnolobium* trees in full bloom (similarly to routine used in the Belgrade survey).

Eventually, owing to communication within CSP networking and prior cooperation (LjS – PN), the detection of *M. sculpturalis* was made possible in neighboring Bosnia & Herzegovina (Nikolić & Bila Dubaić, 2021). In addition to our surveying (throughout the city of Banja Luka), one more B&H location was reported through the CSP, thereby extending our initial study scope to a wider SE European expansion front.

In addition to our field surveys and CSP reports, we continually searched the main international (e.g., GBIF; iNaturalist.org; Observation.org), regional and national internet platforms (including naturalists' online forums and social media-groups) for new or previously unrecognized records of *M. sculpturalis* in Serbia, B&H or other neighboring Balkan countries. Furthermore, the routine scrutiny of recent publications on bee faunistics, invasive bee species and pollination ecology yielded a single additional record from Serbia.

### Other research activities

At various visited locations we collected the bee specimens for population-genetic studies and collected pollen samples for the study of trophic interactions (JBD, MP, JR, PN). We collected bees mostly while they foraged on *Styphnolobium* inflorescences, rarely at nesting places. Typically, we used a standard entomological hand-net, which limited collecting to lower, reachable tree branches, except when additional facilities were available (as in Fig. 2C; foraging sculptured resin bees often concentrate in upper crown portions). Pollen samples were mostly taken from female scopal loads collected while foraging, with only a few from females at nesting settings, or directly from nest cells. All samples were sent to the Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences Vienna, Austria, for further processing (by JL).

Data processing and presentation

As mentioned above, this contribution is focused on presenting the occurrence data gained through all our activities during 2020, based on their relevance and merits for understanding the current state of *M. sculpturalis* expansion in SE Europe. Other results and outcomes were withheld, although sparingly referred to in the text. Specifically, an in-depth elaboration of assessment protocol advances and ensuing analyses of outcomes will be dealt with in separate studies, pending the sufficient surveillance coverage and ample testing performed over an adequate timespan (i.e., several seasons). We report herein on selected survey outcomes and relevant experiences, without going further into methodological details or far-reaching evaluation of the survey results (e.g., abundance assessments, work effort *vs*. recording efficiency estimates, full floral surveillance and resource estimates, etc.). Similarly, we have refrained from in-depth analysis of CSP outcomes and experiences or of the more general aspects of the approach suitability for this study topic (e.g., its comparative strengths and weaknesses in the Serbian/Balkans context), pending the sufficient duration of the endeavour.

For the different types of research activities on *M. sculpturalis* conducted during 2017–2021, we established coordinated thematic databases (by AĆ, JBD, JL). In these we store and maintain extensive sets of data and metadata, comprising detailed primary inputs from all sources, and various kinds of data processing (e.g., diverse calculations and interpretations; for CSP inputs, especially relevant are the means of verifying data accuracy, i.e., species identification and location precision). For the purpose of this paper, we integrated records from 2020 into a summary database, with a selected subset of faunistic and ecological data types: source of record and recorders' details, recording locality/site info (with varying details), altitude and coordinates (with source and accuracy info), date and time of recording, method of recording (with relevant details: in particular, the effort-time of performed assessments), habitats/landscape types, nesting (type, context, etc.), visited plants, with type of recorded interaction (particularly the pollen-gathering), various abundance indices for bees and for plants (including blooming status). In line with the restricted scope of this contribution, herein we have presented a selection of the most essential evidence (summarized in Supplementary material, Table S1), i.e., basic faunistics and detection context data.

Expansion dynamics for the period 2017–2020 is compiled from all available sources and presented in summary maps at the two studied scales: (i) aggregated local occurrences and yearly pattern of detections within the Belgrade area (Fig. 3) are contrasted with distribution and coarse abundance indices of key floral resources, as assessed in 2020 (Fig. 1); (ii) at the regional scale, we have shown all records from Serbia and B&H (aggregated as necessary), complemented with a few most adjacent records from neighboring countries: S Hungary and SE Croatia (Fig. 4). Records from Hungary were specifically added to enable inference of likely regional range extension attained during the seasons of 2019 and 2020, respectively.

We used Google Earth Pro (Google Inc., 2020) for mapping routines, from primary georeferencing of our research data (or other data acquired without coordinates) to verification of location accuracy/consistency from the CSP reports. Furthermore, we used the 'Polygon' tool in Google Earth Pro to define convex hulls of approximate *M. sculpturalis* range and to estimate its hypothetical expansion. We used Google Earth™ Terrain layer to extract the altitude of each documented location. Georeferenced datasets were then imported into QGIS (QGIS Development Team, 2018) for further map processing. Depending on the context and scale, maps depict either exact locations (Fig. 1B, most of Fig. 4), or variously aggregated data; within the Belgrade area (Fig. 1A, Fig. 3) it follows the 'landscape framework' approach from Bila Dubaić *et al*. (2021 [in rev.]; also at: https://srbee.bio.bg.ac.rs/english/m-sculpturalis-2019-survey). Final maps were customized for publication with various picture-editing software.

## Results

During the season of 2020 we established the presence of *M. sculpturalis* at numerous locations in Serbia (mostly in the northern part) and at two locations in Bosnia & Herzegovina. Herewith we present all available records for the two countries since 2017 (from all sources), and we review the most relevant aspects of the *M. sculpturalis* spreading, at two scales: through a detailed survey for the Belgrade area (Fig. 3), and through a summarizing coverage of the current SE European range (Fig. 4). In both cases, we aggregated the primary point-data into operative 'locations' (more strictly standardized for the Belgrade survey), while providing a sufficient level of detail for the newly presented data in the Supplementary material, Table S1. Records prior to 2020 are included from the respective primary sources: Ćetković & Plećaš (2017), Insekti Srbije (2018), Mudri-Stojnić *et al*. (2021) and Bila Dubaić *et al*. (2021 [in rev.]).

### Local scale: a survey of the Belgrade patterns

In 2020 we recorded *M. sculpturalis* at 22 locations across all landscape-urbanistic zones within the core Belgrade area (ca. 19x9 km), 19 as a result of our field survey and three from the CSP reports (Supplementary Material: Table S1, Fig. 3; all Belgrade locations are presented as r=250 m circular sectors). At eight of these recording locations *M. sculpturalis* was also found in 2019, while at seven other locations from 2017–2019 we could not repeat the finding. Most of the records were made on plant inflorescences (almost all on *Styphnolobium*; the single male-based record on *Buddleja*: #3 in Supplementary material, Table S1), three records were associated with nesting activities (two of them in the proximity of blooming *Styphnolobium* trees) and one represents a female collecting resin on a coniferous tree. The few earliest finds (June 29, July 09) coincided with the very early phase of *Styphnolobium* blooming at just a few sites (typically ≤20% of the respective crowns); the last find coincided with nearly finished blooming of the great majority of *Styphnolobium* trees. Positive recordings spanned a period of 50 days (June 29 – August 17), but effectively happened on only 14 days (out of a total engagement of 37 days). Active females were present throughout this period, while males were observed only until July 24 (effectively on five days, at six locations). Generally, the number of recorded specimens was relatively low in most places, rarely exceeding 1–2 per observational unit-time.

Due to the uneven distribution of visited *Styphnolobium* sites (Fig. 1A), different landscape-urbanistic zones were covered with varying surveying effort, and consequently, recording locations are unevenly distributed (2–6 per zone). We recorded *M. sculpturalis* at about 35% of all locations visited during the blooming period; the share of locations with confirmed occurrences varied between zones (26–100%). Despite various logistic constraints, we covered as many as 58 locations within the blooming period, with varying intensity of visitation (frequency and duration per site). Out of a total 159 site visits, 45 were very short (≤2') and/or conducted on trees with relatively few active flowers, thereby with reduced capacity for detecting bees (none yielded any bee record); other visits were fully representative (often >10'). Repeated visits were made to 37 locations during the blooming season: 27 locations were visited 2–4 times, and 10 locations were visited 5–10 times. Nevertheless, only at three locations were we able to detect bees more than once: twice at a site in the BUC zone (over a 28-day interval) and three times at two sites in the PPU zone (over a 17/18-day interval). Overall, we scored only 24 recording events from 19 locations.

Along with the search for the presence/activity of *M. sculpturalis*, we surveyed and quantified *Styphnolobium* trees within 71 analytical r=250 m sectors, totaling roughly 14 $km^2$ (Fig. 1A). In comparison with 2019, we complemented the spatial coverage with 31 new sectors (increase of nearly 78%), comprising about 550 blooming trees; we also recorded 216 additional trees within the sectors assessed in 2019 (a 45% increase). In total, we detected more than 1,250 *Styphnolobium* trees within the survey area (ca. 16x10 km). This is certainly far from a complete inventory, but likely accounts for >95% of trees present within the assessed sectors, and could possibly represent >80% of available trees across several intensively surveyed wider city sections (particularly within the BUC and PUC zones). The earliest scarce blooming we detected in the period June 29 – July 07, at only three locations (all in PUC zone).

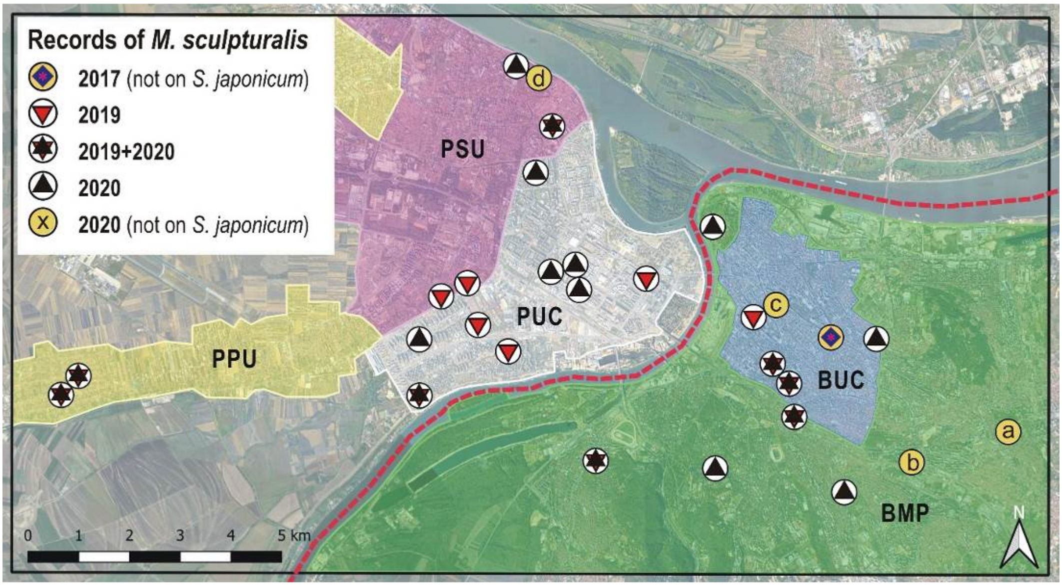


Figure 3. Summary review of documented *M. sculpturalis* occurrences within the Belgrade area in the period 2017–2020. Actual recording localities are aggregated into respective landscape sectors (r=250 m; as explained for Fig. 1). Majority of records represent the bees' foraging activity on blooming *Styphnolobium* trees (*S. japonicum*), exceptions are shown with orange-background circles: 2017 – the first record in Serbia: single male at *Trifolium*; 2020 – "x" in the legend is replaced with respective letter in the map, as follows: a – female collecting resin from a coniferous tree, b – female nesting in a hole in wooden table, c – single male at *Buddleja* blossom, d – female inspecting crevices in a brick pillar (within a line of blooming *Styphnolobium* trees all-around). Locations labelled with a, b, and c represent CSP records. Base map and zonation as in Fig. 1.

Only after July 09–11 did blooming became widespread, so many trees within most of the surveyed sectors were suitable for assessing bee activity. Relatively late-blooming trees (with ≤3% of opened flowers in the period as late as July 16–21) were recorded within at least 11 sectors (19%). Most trees finished blooming soon after August 15–17 (the state when most inflorescences remained with ≤5% active flowers), thereby becoming unavailable as bee forage; a few trees finished blooming as early as July 27–31. Certain *Styphnolobium* locations we managed to survey only outside the blooming period (13 out of 71 sectors were assessed mostly after blooming was over, up to late October). These could not be assessed for bee presence/activity in 2020 but were included in the presentation (Fig. 1A) to provide a more complete floral resources overview for the whole season (as well as for future planning). Distribution of sectors (total *vs*. those assessed in bloom: 71/58) by landscape-urbanistic zones was 15/11 in BUC, 17/14 in BMP, 25/23 in PUC, 11/8 in PSU, 3/2 in PPU.

Regardless of the different phenophases in which we assessed various trees and sectors, we were able to establish that most *Styphnolobium* trees bloomed successfully in the season of 2020. Most of the tree crowns were >95% covered with inflorescences (considering the terminal branches which were in a state that allows blooming), very few had crown coverage of 85–95%, while we recorded only four fully grown trees that did not bloom at all in 2020. Therefore, the floral resources available to *M. sculpturalis* bees were plentiful and almost evenly distributed over the studied sectors for about 30–35 days, though they were greatly reduced and patchily distributed during the early- and late-blooming periods, respectively (each lasting about 10 days, when rare trees with 'outlying phenology' were most important).

Our surveying of other prospective plant genera yielded no record of *M. sculpturalis* in 2020 (the exceptional observation on *Buddleja* was from a CSP). Out of seven initially planned genera, we were only able to sufficiently survey three: *Lavandula, Koelreuteria* and *Buddleja* (Fig. 1B). We had extensive prior experience and knowledge of distribution and phenological suitability of *Lavandula* in Belgrade setting, while for the other two plants our 2020 survey provided the baseline evidence. We surveyed 14 wider locations with *Lavandula* plots (of different sizes and spatial arrangements) and conducted 25 unit-observations in the period June 04 – Sept 03 (on 18 days, but only 12 during favorable blooming conditions); similar to the assessment of *Styphnolobium*, we consider a 'unit-observation' as any minimal duration of targeted observation per unit-location in a single day. We documented 20 locations with more than 140 *Koelreuteria* trees (variously grouped/clustered, from 1–6 to >50 trees per location), and we conducted 28 unit-observations in the period May 30 – July 15 (on 16 effective days). At most locations, the meaningful phase of blooming was reached only after June 10 and it was mostly over after July 05 (only a few late-blooming trees were noted). We surveyed six locations with *Buddleja* bushes (of different sizes, 1–5 separated units per location) and conducted 29 unit-observations in the period June 29 – Sept 04 (on 22 effective days). Throughout this period (and beyond), there were sufficient active *Buddleja* blossoms to justify surveying efforts. Since no bee activity was recorded, we have not provided any more detailed estimates of the available floral resources of these plants; accordingly, no aggregated quantification was attempted, comparable to sector-based quantification for *Styphnolobium* (only 'raw' distributions are shown in Fig. 1B).

As for other initially considered plant genera, our 2020 survey has shown various limitations regarding their utility for *M. sculpturalis* assessment/monitoring, at least for the current situation in the Belgrade area, and thus, we have omitted them from mapping. *Catalpa*: recorded at more than 10 locations (many more are available), but we limited our observations to 6 in the period June 12–29; all observed trees were already in the final blooming phase by June 20, therefore hardly overlapping with the *M. sculpturalis* activity period, at least in 2020. *Wisteria*: recorded at >15 locations, but none of the observed plants showed hardly any blooming after early May, and thus phenologically unsuitable (possibly due to local cultivars; this contradicts the examples documented elsewhere in Europe). *Ligustrum*: none of the numerous locations with various ornamental forms and varieties of this common plant in Belgrade green areas had cultivars that bloom in summer; due to logistic reasons, we could not search for wild *Ligustrum* (likely available in parts of the wider peripheral zones), and this became an even more unrewarding option after we documented a very low activity density of *M. sculpturalis* on optimal floral resources. *Lythrum*: for similar reasons, we largely reduced our engagement to checking the suitability for monitoring on this late-blooming native plant – we made only five observations in August, without positive results; wild stands of this plant are common around small running or standing waters throughout the Belgrade periphery, usually after mid-July, and we could not locate any site with ornamental *Lythrum* stands (ornamental forms were the basis for many recordings elsewhere in Europe or the USA).

As for the installed trap-nests, no nesting of *M. sculpturalis* was detected in any of them, neither by inspecting nor by rearing (relatively few other Hymenoptera were reared from the nests, so this is not of interest to the scope of this paper).

Regional scale: surveying in Serbia (beyond Belgrade) and Bosnia & Herzegovina

Apart from the wider Belgrade area, where we have compiled occurrence data from 29 standardized unit-locations since 2017, we have further documented *M. sculpturalis* presence at 16 other localities in Serbia (Fig. 4). Three of them comprise several 'sub-localities' (seven in Novi Sad, four in Bačka Topola, two in Temerin), totaling 26 unit-locations (which are mostly comparable in size with unit-locations from the Belgrade survey; closer toponyms and/or differing coordinates available in Supplementary material, Table S1). Only two of these localities represent occurrences recorded before 2020: Palić (2018) and Bački Maglić (2019). From Bosnia & Herzegovina we documented the presence of *M. sculpturalis* at two wider localities in 2020 (near Bijeljina and in Banja Luka).

Within some larger localities we detected the bees at most of the surveyed sub-localities (Banja Luka: 5/5; Bačka Topola: 4/4, Novi Sad: 6/9), while at others recording was less successful (Vršac: 1/5, Pančevo: 1/3, Temerin: 1/2). Generally, a significant part of our surveying efforts resulted in 'negative records' – when no activity of *M. sculpturalis* could be detected on blooming *Styphnolobium* trees: we surveyed 19 such sites in Serbia outside Belgrade (in addition to 38 *Styphnolobium* unit-locations in Belgrade). Only two 'negative recordings' were specifically presented in the map: locations in SE Serbia, in the Niš city center, and in the nearby much smaller settlement of Niška Banja, representing our southernmost research area in 2020.

CSP participants in 2020 provided new records from 15 locations in Serbia (three in Belgrade) and one location in B&H. In two cases, reports were cross-posted both to our CSP-network and to the Facebook group "Insekti Srbije" (https://www.facebook.com/groups/insectserbia/), partially available also through the Alciphron portal (https://alciphron.habiprot.org.rs/) (rec_#4 and #32 in Supplementary material, Table S1). A short summary of the CSP reports is provided in Table I. Our field work provided unique records from a further 16 locations in Serbia (other than Belgrade) and five locations in B&H (within Banja Luka); in addition, we visited eight locations to confirm CSP reports. At 11 of these 28 locations, we conducted repeated observations on *Styphnolobium* trees (2–5 times), which resulted in repeated recordings of *M. sculpturalis* at six locations (55%). CSP reports extended over the full two-month period: July 01 – August 31, closely followed by our extended field work outside the Belgrade area: July 05 – Sept 05. Documented phenology of *M. sculpturalis* was generally similar throughout the whole studied area, recording incidence being shifted by just a few days outside Belgrade; exceptions are the records from the southernmost location in W Serbia (#63–64: Skržuti, August 31 – Sept 05).

Overall, we summarized evidence for 61 unit-locations of confirmed *M. sculpturalis* occurrence from the two countries: one from 2017, one from 2018, 15 from 2019 and 53 from 2020. For practical reasons, we mapped recording sites as aggregated into 18 main localities (Fig. 4), plus a more complex presentation of the Belgrade area.

The first detection in Bosnia & Herzegovina (Nikolić, 2020) was based on a nesting event (three females) in an artificial facility (installed for rearing of *Osmia* orchard bees) in early August 2020 in Banja Luka; it was immediately followed by a limited observation survey on *Styphnolobium* trees throughout the city area, generally documenting moderate to high local population abundance. Later, an additional recording location was reported through CSP (Fig. 4; Nikolić & Bila Dubaić, 2021).

For the two blooming seasons, we defined two hypothetical convex hull polygons to depict the minimal range extent of *M. sculpturalis* within the region south of Hungary. Assuming that it was likely established as continuous within the Pannonian and peri-Pannonian lowland area, the estimated range extension was more than doubled, from about 27,000 km$^2$ by 2019 to nearly 56,000 km$^2$ in 2020.

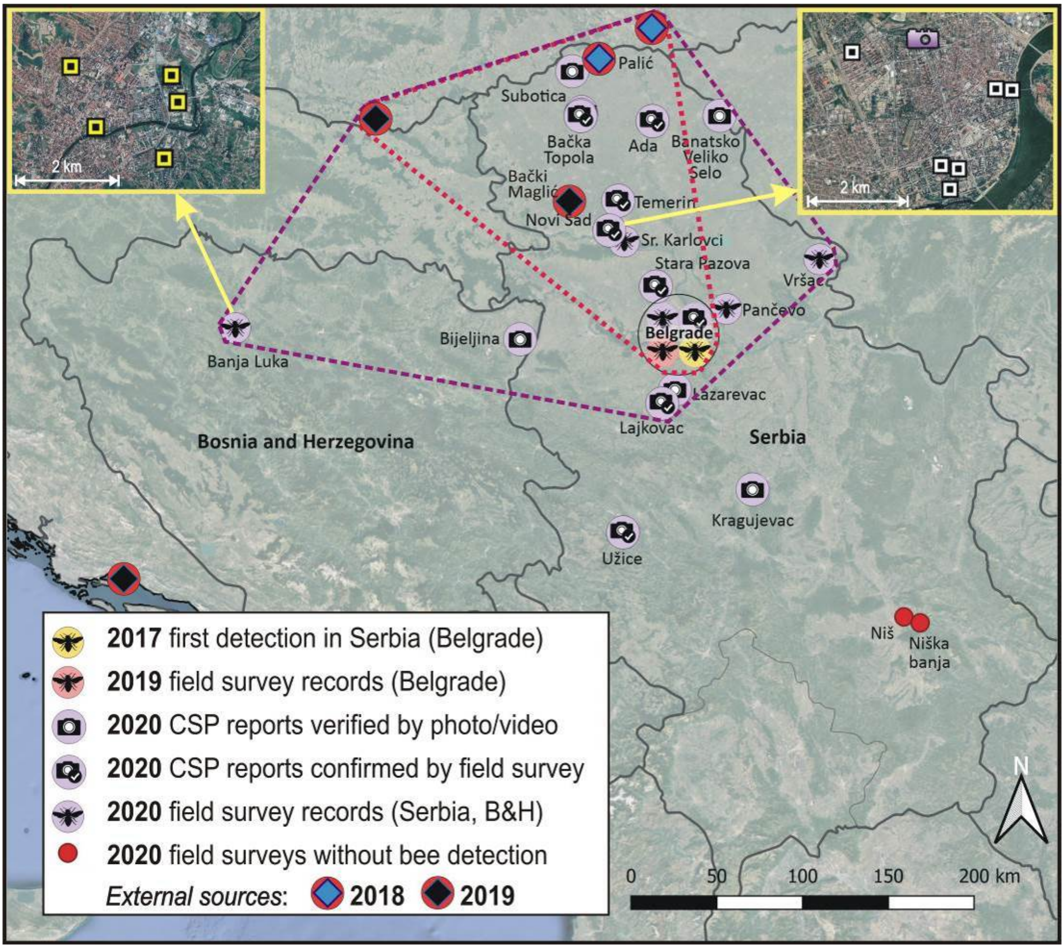


Figure 4. Summary review of documented *M. sculpturalis* occurrences in Serbia and Bosnia & Herzegovina, for the period 2017–2020, by data source and quality. For the Belgrade area, as most intensively surveyed, only the summary of record types is shown (compare with detailed distribution in Fig. 3). Insert-maps show records within the city-areas of Banja Luka and Novi Sad, respectively. The two southernmost of the surveyed locations represent the important outlying 'negative evidence' (bees not detected, despite the effort). From the external sources only two additional records were available from Serbia: Palić in 2018 (Insekti Srbije, 2018), and Bački Maglić in 2019 (Mudri-Stojnić *et al*., 2021). The three most adjacent records to the north and to the southwest depict the documented range extent in the respective countries bordering the survey area: Hungary by 2018–2019 (Rovarok, pókok, 2017–2019; izeltlabuak.hu, 2018) and Croatia by 2019 ('pitrusque', 2019). Two hypothetical convex hulls depict the approximate minimal extent of bee's continuously established range within the area, before the respective blooming seasons: 2019 (dotted/red) and 2020 (dashed/violet). Base-map source: Google Satellite™.

## Other research activities

At a number of visited locations we sampled 88 bee specimens (81 females, seven males) for population-genetic studies, in 21 recording events (i.e., unique locality/date combinations), mostly while foraging on *Styphnolobium* inflorescences (76 specimens including 13 taken by a CSP participant), rarely from nesting settings (12 specimens). Also, we gathered 58 pollen samples in 16 recording events: seven were from nest cells (one nest), the others from scopal loads – 10 from females caught at nesting holes (at two sites), all others were foraging on *Styphnolobium* inflorescences. Ongoing molecular analyses should provide a comprehensive insight into local and regional colonization history and the pattern of population build-up, as well as filling the knowledge gap on floral preferences of *M. sculpturalis* (Lanner *et al*, 2021; Bila Dubaić & Lanner, 2021).

Table. I. The summary of all reports gained through CSP (more details on *M. sculpturalis* reports in Supplementary material: Table S1). Three nesting situations were: in 'bee-hotel' setting (rec#8), in a wooden table (rec#21), and in a semi-withered tree trunk (rec#42). The most unusual case of nesting material were females observed depleting freshly applied grafting wax from the cherry trees (rec#44; Fig. 2A); to our knowledge, this behaviour has not been reported so far, and may represent potential nuisance for the commercial fruit producers.

| | | | |
|---|---|---|---|
| | **Structure of CSP reports** | | |
| **Total** | **Confirmed as *M. sculpturalis*** | **Other insects (bees, wasp, flies)** | **Reports without photo or video** |
| 77 | 16 (21%) | 51 | 10 |
| **Confirmed reports of *M. sculpturalis* (16)** | | | |
| | **Area** | | |
| Belgrade | Serbia (except Belgrade) | Bosnia & Herzegovina | |
| 3 | 12 | 1 | |
| | **Sex of reported individuals** | | |
| Females | Males | Both (additional reporting) | |
| 12 | 4 | 1 | |
| | **Observation context** | | |
| **Foraging on flowers** | | **Collecting the nesting material** | |
| *Sohphora* | *Buddleja* | Resin (conif. trees) | Grafting wax (in orchard) |
| 4 | 1 | 3 | 1 |
| **Nesting** | **Other situations** | | |
| | Alive - indoors | Dead - indoors | Dead - outdoors |
| 3 | 1 | 1 | 2 |

## Discussion

### Local scale: a survey of the Belgrade patterns

As in the previous season, our 2020 Belgrade survey confirmed the strong association of successful detections of *M. sculpturalis* with the availability and adequate assessment of *Styphnolobium* trees in bloom. During the season of 2020 we greatly extended our surveying efforts, not just regarding the spatial coverage of *Styphnolobium* floral resources (+78% of unit-locations, +158% of surveyed trees), but also with the inclusion of other prospective plant genera, with an ample phenological span and far more intensive field work. However, we managed to detect *M. sculpturalis* at only 19 locations associated with *Styphnolobium* across Belgrade. Another three records (all from the CSP) were not related to *Styphnolobium*. Our results show a modest increase of 36%, compared to the 14 locations recorded in the limited survey in 2019 (Bila Dubaić *et al*., 2021 [in rev.]; Fig. 3). Considering only the phenologically suitable survey period of 50 days of blooming *Styphnolobium*, the recording success was only about 35% in 2020 (19 out of 58 locations), compared to 88% in 2019 (14 out of 16 suitable locations, surveyed only within the last 8 days of scarce

*Styphnolobium* blooming). Other parameters of detecting efficiency also indicated very low population level, e.g., number of recording events and recorded specimens, compared with the overall intensity of surveying. The low population abundance of *M. sculpturalis* was further corroborated by the lack of nesting in any of the installed nesting facilities.

Bila Dubaić *et al*. (2021 [in rev.]) have shown how a strongly reduced blooming of the key food plant (*Styphnolobium*) in 2019 promoted strong local concentration of bee activity around scarce resources, thereby enabling easy and mass recording. This was contrasted with poor detectability across northern Serbia during 2017–2018 due to the 'dilution effect'. The effect occurs when super-abundant floral resources induce a very low average activity density of bees per 'unit-resource'. In contrast with the extreme situation in 2019 (blooming reduced to about 6% of the average intensity), the 2020 season had highly successful *Styphnolobium* blooming, providing exceedingly abundant and evenly distributed key food resource. The overall poor recording success in 2020 indicates that we have witnessed a repeated dilution effect on the local bee population. It is possible that, at least in the Belgrade area, populations of *M. sculpturalis* were additionally reduced due to diminished reproduction during the food-limited summer of 2019. It is a well-known phenomenon that the interseasonal variation of key food resources may affect both the local bee reproduction and the frequency of occurrences (cf. Tepedino & Stanton, 1981; Crone, 2013). This could create the alternation of concentration and dilution effects, leading to the dynamics of activity density observed for *M. sculpturalis* in Belgrade during 2017–2020. Remarkably, *Styphnolobium* seems to follow the 'alternating' or even markedly 'irregular bearing' pattern, otherwise recorded in numerous tree taxa belonging to widely different plant families (Monselise & Goldschmidt, 1982). It is of great relevance to future monitoring efforts to account for the variable blooming pattern of *Styphnolobium*, as the most important food resource in our 2019–2020 surveys.

The total of 29 different locations for the period 2017–2020 (Fig. 3) indicates widespread presence that is seemingly without any notable pattern. The current recording tally represents an evidence base that is still inadequate to reveal possible spatial differences, e.g., the effects of varying habitat compositions or wider urban environmental gradients. Comparison of recording success between the landscape-urbanistic zones may indicate some meaningful differences. The highest share (100%) was maintained in the PPU zone in both seasons (2019–2020). The zone represents an isolated peripheral settlement surrounded by wide areas of inhospitable agricultural land, with *Styphnolobium* trees restricted to just a few points. Therefore, repeated occurrences of *M. sculpturalis* at both PPU locations, including a high incidence of repeated findings during 2020, further support the idea that localized resource concentration greatly improves detectability (Bila Dubaić *et al*., 2021 [in rev.]). The lowest recording share was in the PUC zone (26%), where we managed to survey the largest number of *Styphnolobium* units with almost 62% of all the trees detected in the Belgrade area. In the remaining three zones, we had almost uniform recording success (36–38%), slightly above the average for the Belgrade area (35%). Despite the seemingly 'averaged state' of these simple metrics, indicating similar population patterns, the real situation was probably neither uniform nor representative for straightforward interpretation. In the most heavily urbanized BUC zone, we had quite high intensity and spatial density of surveying, which yielded rather poor outcomes: records were mostly peripheral (near the surrounding BMP zone), hence the repeated records (2019–2020) at two locations may not be considered as remarkable as in case of PUC. The two remaining zones (BMP and PSU) seem to be insufficiently assessed as regards the sparse and uneven location coverage and undersampled resources.

Nonetheless, on a coarser scale, recording success within the Pannonian *vs*. Balkan sections (Fig. 3; see details at: https://srbee.bio.bg.ac.rs/english/m-sculpturalis-2019-survey) appears to be 'stabilized' around similar average values (33% and 36%, respectively). Further testing is needed to explore the relevance of this tentative measure of 'effort *vs*. coverage index': could the detection success of about 35% (with suitable coverage) provide a minimal target value for reaching the meaningful estimates of local activity density of bees at low initial population levels. In parallel, we should further test what is the representative number of unit-locations over a wider spatial extent (at various scales) needed to enable reliable monitoring with minimal/feasible effort. These preliminary indices justify the efforts to provide extended and more accurate quantification of floral resources on a wider scale. So far, our initial r=250 m grid framework seems to be a highly practical and operative approach for exploratory field studies. However, coarser scales are probably more suitable for assessing the activity patterns and preferences of such a large and highly vagile bee. It is particularly challenging to deal with the phenological dynamics and variability (both of bees and of target plant taxa) at the respective landscape scale, i.e., to 'capture' realistic bee activity and interaction indices within the shifting availability of floral resource. Our intensive surveying of an exceedingly abundant *Styphnolobium* floral resource with ample phenological coverage throughout the Belgrade area (Fig. 1A) provided a sound baseline for comprehensive future estimates at a wide range of spatio-temporal scales.

Besides the key floral resource, we extended the survey to other possible foraging plants. However, unlike in other studies across Europe, it is remarkable that we had so very few detections of *M. sculpturalis* foraging on other plants. In only two cases over the period 2017–2020 were males observed feeding on plants other than *Styphnolobium* (Fig. 3). Bila Dubaić *et al*. (2021 [in rev.]) tentatively associated this curiosity with the early phase of colonization in Serbia (Belgrade), characterized by population abundance being too low to support the 'spill-over' effect from the principal pollen-source plant. Accordingly, some of these additional plant genera are expected to serve as important complementary 'monitoring plants', once the local bee abundance reaches a sufficient level.

For example, *Lavandula* is among the most frequently visited plant taxa in the European range, second only to *Styphnolobium* (cf. Ćetković *et al*., 2020: unpublished study); it is even ranked first in some country accounts, such as France (Le Féon *et al*., 2018) and Italy (Ruzzier *et al*., 2020). So far, we have no observations of *M. sculpturalis* on this plant, despite its widespread presence in Belgrade. In addition to the still low bee population levels, some other reasons possibly reduce the suitability of *Lavandula* in the Belgrade context: improper management regime on most public floral sites and/or unsuitable cultivar selection. Over an extended period (>8 years, unpublished observations), the blooming of *Lavandula* in Belgrade was usually much reduced or even finished as early as July 10–15 (when complete trimming often being practiced), while a meaningful blooming extent recovers only in a few places, sometime in late August. Therefore, in the Belgrade setting, *Lavandula* could be considered attractive for *M. sculpturalis* only before mid-July, and therefore useful for comparative surveying in the early phase of seasonal activity.

Of other prospective plants, *Koelreuteria* is largely comparable with *Lavandula* in the phenological aspect, and therefore potentially useful for surveying in the same seasonal phase, particularly after mid-June. It is also a widespread and abundantly planted ornamental tree across Belgrade (Fig. 1B), representing a plant of different life-form and geographic origin from *Lavandula*, while similar to *Styphnolobium* in these respects. Finally, our 2020 survey documented that the third compared plant, *Buddleja*, could be useful as an alternative or 'control' monitoring unit, since it blooms continuously during most of the seasonal activity of *M. sculpturalis* females and overlaps with the other three plant genera in an important period: late June – mid-July. It is currently not so abundant and widespread in Belgrade, but its distribution seems sufficient for comparative analyses. All four considered plants are of special interest also for studying the relative preferences and possibly altered interactions among some common summer bees (genera *Apis*, *Bombus*, *Anthidium*, *Xylocopa*, native *Megachile*, etc.).

The lack of records from two of the three compared plants largely corresponds with our still poorly documented early phenology of *M. sculpturalis* in Belgrade. Its activity is expected to begin by mid-June, based on the flight period recorded elsewhere in Europe: early June – mid-September in Italy and France (Ruzzier *et al*., 2020; Le Féon *et al*., 2021); mid-June – late August in Hungary (Rovarok, pókok, 2017–2019; izeltlabuak.hu, 2018); thus, at least two or three of the earliest weeks have not yet been documented. This may also in part explain the unrealistically small share of males in our recordings in Belgrade (which was similar elsewhere across our study area). The activity of males could precede that of females by about 10–15 days (Kakutani *et al*., 1990) while the effective sex ratio could be as much as 72% male-biased, based on total brood emergence (Sasaki & Maeta, 2018). Extensive worldwide evidence (cf. Ćetković *et al*., 2020: unpublished study) clearly shows a predominance of male visitations to all three alternative plant genera from our survey. Therefore, we hypothesize that a more realistic sex ratio will be evidenced when a higher abundance of *M. sculpturalis* would allow for an observable 'spill-over' effect from the mass-blooming *Styphnolobium*. Similarly, we still lack evidence for approximately the final two weeks of female foraging/nesting activity (late August – early September).

Regional scale: distribution in Serbia (beyond Belgrade) and Bosnia & Herzegovina

We established that by the season of 2020 *M. sculpturalis* had colonized more than a third of Serbia and arguably a quite extensive tract of northern Bosnia & Herzegovina; it was recorded within 19 aggregated localities (Fig. 4), only three of them being documented before 2020. A detailed review for the period 2017–2020 is based on evidenced occurrences within 61 unit-locations. The importance of *Styphnolobium* for the detection of *M. sculpturalis* is further emphasized at this scale: at only 13 unit-locations was the presence of this key food plant not documented (Supplementary material, Table S1). Accordingly, the temporal span of all recordings throughout the region was strictly defined by the phenology of blooming *Styphnolobium* trees (June 29 – September 05).

Based on the currently documented distribution and the pattern of detection dynamics during 2017–2020, we assume that the Pannonian part of Serbia (Province of Vojvodina) was probably fully colonized well before 2020, despite the initial paucity of records. Such a pattern was first suggested based on findings from the eastern Pannonian Plain that were available in 2019 (Bila Dubaić *et al*., 2021 [in rev.]), and is analogous to documented dynamics of spread in some other countries of Europe (cf. phase maps at: Ćetković *et al*., 2020). *M. sculpturalis* is now fairly well established, frequently encountered and numerous across Vojvodina. The average recording success per visited *Styphnolobium* site was much higher than in the Belgrade area (ca. 50% *vs*. 35%), despite considerably less intensive surveying. This could be indicative of both the higher population levels (due to earlier local establishments) and/or more efficient detection due to favorable and spatially restricted situations. We have found it in a range of mostly urban environments, including a few larger cities (Novi Sad, Vršac, Subotica) and several smaller towns, but also in some rural settlements. The region is characterized by flat terrain, mostly dominated by agricultural land use and hence generally unsuitable for this bee species due to the lack of *Styphnolobium* or other proven pollen-source plants. However, the area is interspersed with numerous settlements (often less than 10–15 km apart) and *Styphnolobium* is present in many of them. It was generally widely planted in all types of settlements in Serbia as both an ornamental and melliferous species, and this situation could have promoted an easy expansion of *M. sculpturalis*, in a kind of 'steppingstone' fashion. All these results further corroborate the suggested 'sneaking distribution scenario' for the introduction of *M. sculpturalis* into Serbia (Bila Dubaić *et al*., 2021 [in rev.]): a continuous southward spreading from NE Hungary (instead of a long-distance jump into Belgrade). Future molecular studies on the genetic structure of the Serbian and other eastern European populations should provide a clearer picture of possible colonization routes (Lanner *et al*., 2021).

Occurrences across the lowland-to-hilly peri-Pannonian zone, from NW Bosnia through central Serbia, are still sporadic, arguably indicating the ongoing widely frontal expansion, southwards from the Pannonian Plain. This tentative expansion zone now spans ca. 250 km W–E, from Banja Luka, through Bijeljina to Lajkovac. The westernmost Bosnian records are about 137 km SW linear distance from the closest known record in southern Hungary (of 2019; cf. Rovarok, pókok, 2017–2019). The alternative sources could have been populations from the Belgrade area and/or Vojvodina (records of 2017–2019), or those from Slovenia (records of 2018–2019), both at about a 250 km linear distance (to the east or to the west, respectively). Smaller distances from the two Croatian coastal records (150–180 km to S/W) are probably irrelevant in this context since dispersal across the Dinaric Mountains range seems much less likely. Notably, the recording in Banja Luka, made on a single day, was extraordinarily successful per visited *Styphnolobium* site (100%), and *M. sculpturalis* was fairly abundant, indicating a much earlier local establishment (Nikolić & Bila Dubaić, 2021). Based on the assumption that spreading was probably continual and unlimited across the lowlands, including across NE Croatia (wherefrom no records are available), the range extension within the lowland area south of Hungary is estimated to have likely doubled between the seasons 2019 and 2020 (Fig. 4).

There are only two scattered records more southerly, in central to western hilly-mountainous areas, indicating that spreading into the core of the Balkan Peninsula is taking place more slowly and not continuously: ca. a 95–125 km linear distance was reached in at least three seasons (since the first Belgrade find in 2017). Further south, extensive and repeated observations in Niš and in Niška Banja during July – August at two sites with numerous *Styphnolobium* trees in full bloom yielded no activity of *M. sculpturalis*, indicating that bee expansion has not yet reached the area (or that the population density is still too low for detection).

With respect to the entire temporal span of recorded *M. sculpturalis* activity, the single southernmost location of Skržuti (near Užice; Supplementary material, Table S1: #63–64) represented a notable exception. In this area we evidenced considerable activity of *M. sculpturalis* as late as August 31 – September 05 (and collected 13 females and four males). All individuals were in fairly good condition (hence, recently emerged) and were intensively foraging on a *Styphnolobium* tree in full bloom. Probably the local bee activity lasted for at least 1–2 weeks after our surveying, while in the rest of the region we documented only a much-reduced activity after mid-August (the last find was on August 22). This was also the highest (512 m a.s.l.) of all records in SE Europe (in our dataset, *M. sculpturalis* is restricted to the lowlands: 75–232 m a.s.l., mean 118 m; cf. Supplementary material, Table S1), but the ecological difference in altitude alone may not explain such a remarkable delay in phenology. However, this small rural settlement is situated within the wider mountainous region of SW Serbia, dominated by the vast nearby plateau of Pešter (average height ca. 1,000 m a.s.l.), and renowned for extremely low winter temperatures. Hence, we attribute this shift to the extraordinary climatic effects of regional topography, affecting similarly the bee species and its key food-plant. Intensive foraging (and nesting activity) of *M. sculpturalis* so late in September has not been documented so far in Europe but is known from northern areas within its native range in Japan (Sasaki & Maeta, 1994). Otherwise, the record is remarkable for its remote position, away from the important traffic routes and from other documented *M. sculpturalis* occurrences. It is situated in wider semi-natural surroundings, with probably only a scattered distribution of the relevant floral resources.

During the first three years of its documented presence in Serbia (2017–2019), detections of *M. sculpturalis* were scattered and accidental, making a time-intensive field survey across the wide geographical area unfeasible. Our pioneering CSP proved to be fairly effective and suitable for this regional scale in that the majority of observations of *M. sculpturalis* outside the Belgrade area were initially made by citizen scientists. Despite the small number of accurate reports, it covered a remarkable spatial extent (ca. 250x130 km, encompassing a convex polygon of >23,000 km$^2$). To improve the coverage of *M. sculpturalis* range dynamics and habitat affinities, in future efforts we need to enhance the engagement of people who live in (or visit) rural, semi-natural or natural areas. So far, we have compiled records from only six such locations (from all sources). Another aspect that could be improved is the low accuracy rate of identifications made by CSP participants (21%), as compared with e.g., bumblebee surveys in the UK (40–60%) (Falk *et al*., 2019). This clearly emphasizes the need for professional verification of species identifications (Soroye *et al*., 2018; MacPhail *et al*., 2020), even in the case of a bee with such a remarkable habitus (Fig. 2D). Generally, tailored CSPs and other forms of general public involvement are confirmed approaches for tracking the expansion of *M. sculpturalis* across Europe (Le Féon *et al*., 2018; Lanner, 2018–2019; Lanner *et al*., 2020a; Ruzzier *et al*., 2020; www.beeradar.info). However, these must be accompanied with well-designed and focused research by professional bee experts in order to establish a much-needed thorough scientific foundation for future monitoring and management of this potentially troublesome species.

## Acknowledgements

We want to thank all the participants of the CSP (>60) who enthusiastically took part in our research, and in particular, those who provided exact information on *M. sculpturalis* occurrences used in this paper: Milica Perić, Matija Zloporubović, Varga Balázs, J.M., Momčilo Adamović, R. Samurović, Dragana Predojević, Ivana Jurić, J.S., Kristina Simić, Snežana Vujković Lamić, Bozsóki Róbert (representing the citizen organization "Zeleno ostrvo Ada"), Gergely József, Todor Radović, Nikola Simanić, Predrag Kovačević and Mitar Stevanović (some names withheld upon request); see also at: https://srbee.bio.bg.ac.rs/azijska-pcela-smolarica/azijska-pcela-projekat-ucesce/gra%C4%91ani-koji-su-dali-svoj-doprinos-na%C5%A1em-projektu.
Special thanks go to participants who contributed further with extended observations of nesting activities (Samurović family; B. Varga), and to those who contributed also by collecting the bee samples (M. Stevanović). The contributions of various organizations (including media) and internet-based platforms (soc-media/groups) to promoting and/or disseminating news about our research were also of great importance. We are grateful to colleagues and friends who participated in installing the nesting facilities around the Belgrade area: Katarina Bjelanović, Katarina Breka, Jelisaveta Čkrkić, Milica Čkrkić Matijević, Maja Dakić, Aleksandra Penezić, Ivan Stojanović, Milja Vuković and Miloš Zdravković. Various research facilities, logistics and consumables were partly provided through the extended funding framework of the Faculty of Biology (University of Belgrade), from the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract: # 451-03-68/2020-14/200178).

## Supplementary material

Supplementary material, Table S1 (PDF): Records of *M. sculpturalis* from Serbia and Bosnia & Herzegovina during 2020 (https://doi.org/10.5281/zenodo.5569521)

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# ДАЉА ЕКСПАНЗИЈА АРЕАЛА ВЕЛИКЕ ПЧЕЛЕ СМОЛАРИЦЕ (*MEGACHILE SCULPTURALIS*) У СРБИЈИ И БОСНИ И ХЕРЦЕГОВИНИ

Јована Била Дубаић, Јована Раичевић, Милан Плећаш, Јулиа Ланер, Петар Николић, Владимир Жикић, Љубиша Станисављевић и Александар Ћетковић

## Извод

Велика пчела смоларица (*Megachile sculpturalis*) је прва неаутохтона врста пчеле у Европи, пореклом из источне Азије. Њено континуирано ширење (од кад је откривена у југозападној Европи, 2008–2010) резултирало је дистрибуцијом која тренутно обухвата готово 2.800 x 1.100 км (по географској дужини, односно, географској ширини), у оквиру јужне и средње Европе. У југоисточној Европи је потврђена од 2015. (у североисточној Мађарској), а затим у северној Србији и широм источне Панонске низије (2017–2019); последње је нађена у северозападној Босни и Херцеговини (2020).

У глобалним размерама изражена је све већа забринутост због растућег броја интродукција алохтоних врста пчела. Због тога расте и интерес за бољим разумевањем образаца и процеса који утичу на њихово успешно колонизовање нових простора, посебно због потенцијалне инвазивности. Најважнији негативни ефекти инвазивних алохтоних пчела могу се испољити у односу на популације аутохтоних врста, као и на различите категорије интеракција повезаних са опрашивањем. У литератури о интродукцији *M. sculpturalis* широм Европе, више пута је истицана потреба за праћењем („мониторингом“) ове „инвазије“, али тренутно не постоје никакви протоколи за процену потенцијалних утицаја, као ни других релевантних параметара везаних за успешност колонизовања. На основу наших истраживања спроведених током 2017–2019. године на подручју Београда, предложен је „радни концепт“ за свеобухватно праћење *M. sculpturalis*, заснован на квантитативној процени популационих трендова ове пчеле у односу на ресурсе кључне биљке хранитељке. Овај иницијални концепт сада треба унапредити, проширити и тестирати, у односу на различите просторно-временске скале истраживања или потребе различитих режима будућег праћења. Зато смо током 2020. значајно проширили опсег истраживања, на две просторне скале. На ЛОКАЛНОЈ скали, за подручје Београда, настављено је интензивно праћење и процена бројности, те проучавање биономије и локалне дистрибуције *M. sculpturalis* (у односу на градијенте станишних услова у урбаној средини); паралелно је вршена евалуација ширег сета релевантних биљака и њихових интеракција, као потенцијалних ресурса хране али и „референтних јединица“ за регистровање активности смоларице. На РЕГИОНАЛНОЈ скали проучавана је дистрибуција и динамизам ареала ове врсте широм Србије и Босне и Херцеговине, као референтног „пресека стања“ експанзионог фронта у југоисточној Европи; ово је укључило шири обухват њених еколошких преференција у односу на различите типове станишта и животних услова. Ради ширег и ефикаснијег обухвата студије, покренут је, као пионирски концепт, наменски „пројекат грађанске/волонтерске науке“ (citizen science project), фокусиран на регистровање присуства врсте широм Србије и региона, што је омогућило значајну географску покривеност истраживања (упркос релативно скромном броју тачних дојава).

Током истраживања на подручју Београда врста је забележена на нешто већем броју локација него у 2019. години (+36%), али је значајно смањена ефикасност регистровања (35%, у односу на 88% у 2019), упркос знатно повећаном интензитету и обухвату истраживања. Ово је додатно потврдило

значај феномена алтернирања „ефекта концентрације“ и „ефекта разређивања“ на детектабилност ове пчеле, изазваног међусезонским варирањем кључних извора хране, што значајно утиче и на динамику популације смоларице. Потврдили смо наглашену везу између ефикасности детекције и доступности кључне биљке хранитељке – софоре (*Styphnolobium*), посебно имајући у виду варијабилност њеног цветања између сезона; ове релације, потврђене на обе скале истраживања, од велике су важности за дефинисање концепта праћења. Фенолошки опсег регистроване активности *M. sculpturalis* (>70 дана) блиско се поклапа са фенологијом цветања софоре током 2020; реални опсег активности на нивоу региона је свакако шири, делом условљен и локалним модификацијама климе услед наглашеног рељефа. Са једним изузетком, практично нисмо имали налазе на другим испитиваним биљкама. Регионална експанзија *M. sculpturalis* у периоду 2017–2020. документована је у склопу детекције на 19 ширих локација, од којих је на 16 врста први пут регистрована у 2020. години. *M. sculpturalis* је сада посебно добро заступљена у панонском, а нешто слабије у перипанонском подручју Србије и БиХ, где се приближно процењени опсег ареала вероватно удвостручио између 2019. и 2020. године. Даље на југ смоларица је нађена на свега пар локација, што указује на спорије ширење врсте кроз брдско-планински део Балкана. У целини, налази су претежно били из градских средина или других типова насеља, тек око трећине потиче из полуприродног или претежно пољопривредног окружења.

Received: September 18th, 2021
Accepted: September 28th, 2021