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A threefold plant specialist – distribution, habitat requirements and nesting biology of the rare leafcutter bee *Megachile genalis* in the eastern Swiss Alps (Hymenoptera, Megachilidae)

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Abstract

The leafcutter bee *Megachile genalis* Morawitz, which nests in thick, erect and usually hollow plant stems, is rare and endangered throughout Europe. In Switzerland, it was recorded only six times in the Grisons between 1932 and 2019. In order to create the necessary basis for the conservation of this rare bee, its distribution, habitat requirements and nesting biology were investigated in the eastern Swiss Alps by systematically searching for its nests, by DNA metabarcoding of the cell-building material, by analysing the pollen contained in abandoned brood cells and larval faeces, and by deducing aspects of the species' nesting biology from collected old nests. A total of 141 new and 64 old nests were discovered at elevations between 600 m and 1270 m in – with decreasing frequency – the Albula Valley, the Lower Engadine, the Domleschg and the Ruinaulta. The nests were exclusively built in living stems of *Peucedanum verticillare* (Apiaceae), an up to 2–3 m tall umbellifer that grew on fallow land, road and railway embankments, scree slopes, ruderal areas and forest clearings. The leaf fragments used by the females of *M. genalis* to construct the brood cells in 20 selected nests were largely from shrubs and trees of the Rosaceae (e.g. *Rosa*, *Rubus*, *Prunus*, *Sorbus*), rarely also from *Corylus* (Betulaceae). The pollen in 65 brood cells was exclusively collected on thistles (e.g. *Arctium*, *Carduus*, *Cirsium*) and other Asteraceae. The nests were characterised by a surprisingly high variability in their architecture. They consisted of i) an approximately 6 mm x 9 mm large nest opening gnawed by the female bee at a height of 22–217 cm above ground with her mandibles, which are well adapted to penetrate the hard stem walls due to their enlarged base indicating strong adductor muscles and the short and chisel-like shape; ii) a 5–25 mm thick nest plug built 1.5–45 cm below the nest opening from pith particles, leaf fragments and/or grass fibers; iii) 1–14 brood cells usually arranged in one cigar-like series and lying 11–99 cm below the nest opening; and iv) a facultative 4–40 mm thick basal plug consisting of pith particles and/or leaf fragments. Brood mortality was high: at least 56% of 284 brood cells were unsuccessful due to parasites, mould or unknown reasons, and reproduction completely failed in almost half of 46 nests. The most harmful brood parasite was *Melittobia acasta* Walker (Hymenoptera, Eulophidae), which infested 19% of the brood cells and 28% of the nests. Measures to conserve the population of *M. genalis* in the eastern Swiss Alps should focus on the conservation and propagation of suitable habitats for *P. verticillare* in close vicinity to Asteraceae-rich areas and on the promotion of thistles.

Key Words

Anthophila, Apiformes, *Coelioxys inermis*, conservation, *Exeristes roborator*, *Lasiambia*, mandibular strength, metabarcoding, pollen analysis, *Trichodes apiarius*, *Xylophrurus augustus*

Introduction

Leafcutter bees of the genus *Megachile* (Megachilidae) use fragments of green leaves or, more rarely, flower petals to build their brood cells, which they either hide in preexisting cavities (insect borings in dead wood, hollow stems, rock and soil crevices, hollow spaces under stones) or in self-excavated burrows in dead wood, pithy stems or loose soil (Praz 2017; Westrich 2018). According to current knowledge, most Central European *Megachile* species appear to be rather flexible in the selection of their nesting sites. One exception is *M. genalis* Morawitz, which constructs its nests exclusively in erect stems of various thick-stalked plants (Ruhnke 1998, 2000; Westrich 2018).

Megachile genalis is very rare throughout Europe and red-listed in Belgium, the Czech Republique, Germany, Slowakia and Switzerland (Feráková et al. 2001; Westrich et al. 2011; Straka and Bogusch 2017; Drossart et al. 2018; Müller and Praz 2024). In Switzerland, it is one of the rarest bee species. It was recorded only six times in the Grisons before 2023, namely in the Lower Engadine in 1932, 2004 and 2013 and in the Albula Valley in 2016, 2017 and 2019. Despite a targeted search in 2018 in the frame of the elaboration of a red list of bees in Switzerland, *M. genalis* was not rediscovered in the Lower Engadine, rendering the Albula Valley the only region in Switzerland where the species was assumed to occur today.

The main objectives of the present study were to clarify the current distribution and abundance of *M. genalis* in the Grisons, to investigate the species' requirements for nesting site, nest-building material and host plants and to get some insights into its poorly known nest architecture. The results should create the necessary basis for the conservation and promotion of this rare bee species at its current sites of occurrence in the eastern Swiss Alps.

Methods

Bee species

Megachile genalis, which belongs to the subgenus *M.* (*Megachile*) (Praz 2017), is a 10–14 mm long bee species distributed from Western Europe via Turkey to easternmost Russia and Japan (Ascher and Pickering 2020; Fig. 1a). In Central Europe, it is a univoltine, late-flying species active from mid-June to early September (Westrich 2018), while in warmer parts of eastern Europe it may have two generations (Wiesbauer 2023). A typical character of the females are the basally strongly inflated mandibles not found in any other European *Megachile* species (Fig. 1b–d). The species builds its nests in erect, thick and usually hollow, rarely pithy stems of various plants (Dudich 1884; Henschel 1888; Buysson 1902; Friese 1923; Grandi 1954, 1957; Dorn and Weber 1988; Ruhnke 1998, 2000; Westrich 2018; Sobieraj-Betlińska 2021; Tischendorf 2021; Wiesbauer 2023). In contrast to all other stem-nesting bee species of Europe, living rather than dead stems are used as nesting sites (Ruhnke 1998, 2000; Westrich 2018; Wiesbauer 2023). Nests of *M. genalis* are easily recognized by the usually oblong-oval shape of the nest opening, which is gnawed by the females into the stem (Figs 1g, h, 5a–d). The nests contain several brood cells, which lie inside the stem in a linear series and are nested into each other forming a cigar-like structure (Fig. 6a, c, h, i). For the construction of the brood cells, the females use roundish to oval leaf pieces (Figs 1g, 6e), which are cut out on green leaves. The brood cells are supplied with a mixture of pollen and nectar supposed to be collected mainly on Asteraceae (Ruhnke 1998, 2000; Westrich 2018; Wiesbauer 2023; Fig. 1a, h). The winter is spent as a prepupa inside a self-spun cocoon within the brood cell (Fig. 6g).

Study area

The main study area was located in the Parc Ela (Grisons) in the eastern Swiss Alps and encompassed the Albula Valley between Alvaschein in the west and Davos Wiesen in the east (Fig. 2). Within the Albula Valley, 16 subplots ranging in area from 4 ha to 53 ha at altitudes between 900 m and 1400 m were selected to search for nests of *Megachile genalis*. These subplots were primarily chosen based on the frequency of sun-exposed fallow land expected to contain plant stems suitable for nesting by *M. genalis*, such as unmown edges along forests and hedges, uncultivated farmland, road and railway embankments or forest clearings. After the fieldwork had revealed that *M. genalis* nests in stems of *Peucedanum verticillare* (L.) Mert. & W. D. J. Koch (Apiaceae) in the Albula Valley (see Results), four additional study areas in the Grisons with known populations of *P. verticillare* were selected (Fig. 2), i.e. the Lower Engadine between Ardez and the border with Austria (one subplot of 465 ha at altitudes between 1000 m and 1400 m), the Domleschg (five subplots ranging from 29 ha to 119 ha at altitudes between 600 m and 1100 m), the Ruinaulta (two subplots of 47 ha and 88 ha at altitudes between 600 m and 850 m) and the surroundings of Chur (two subplots of 26 ha and 33 ha at altitudes between 550 m and 1000 m). The selection of the subplots in these four additional areas was based on data provided by the National Data and Information Center on the Swiss Flora (info flora) on the occurrence of *P. verticillare* in Switzerland after 1999.

Nesting biology

In the 26 subplots of the five study areas, nests of *Megachile genalis* were searched for during a total of 14 days and 105 hours from 23 July to 5 October 2023. Both living and dead and both hollow and pithy stems of all plant species and individuals with an outer stem diameter of at least 10 mm near their base were checked for the presence of the characteristic nest openings, which differ from those of other stem-nesting aculeate Hymenoptera, such as *Hoplitis tridentata* (Dufour & Perris) (Megachilidae), by their size and their oblong-oval shape (Fig. 5a–d). Due to the relatively large body size and the relatively thick cell walls consisting of several layers of leaf fragments, *M. genalis* is not expected to nest in stems with an outer diameter of less than 10 mm, which is confirmed by Ruhnke (1998, 2000), who recorded a minimum outer stem diameter of 13.5 mm in 36 nests.

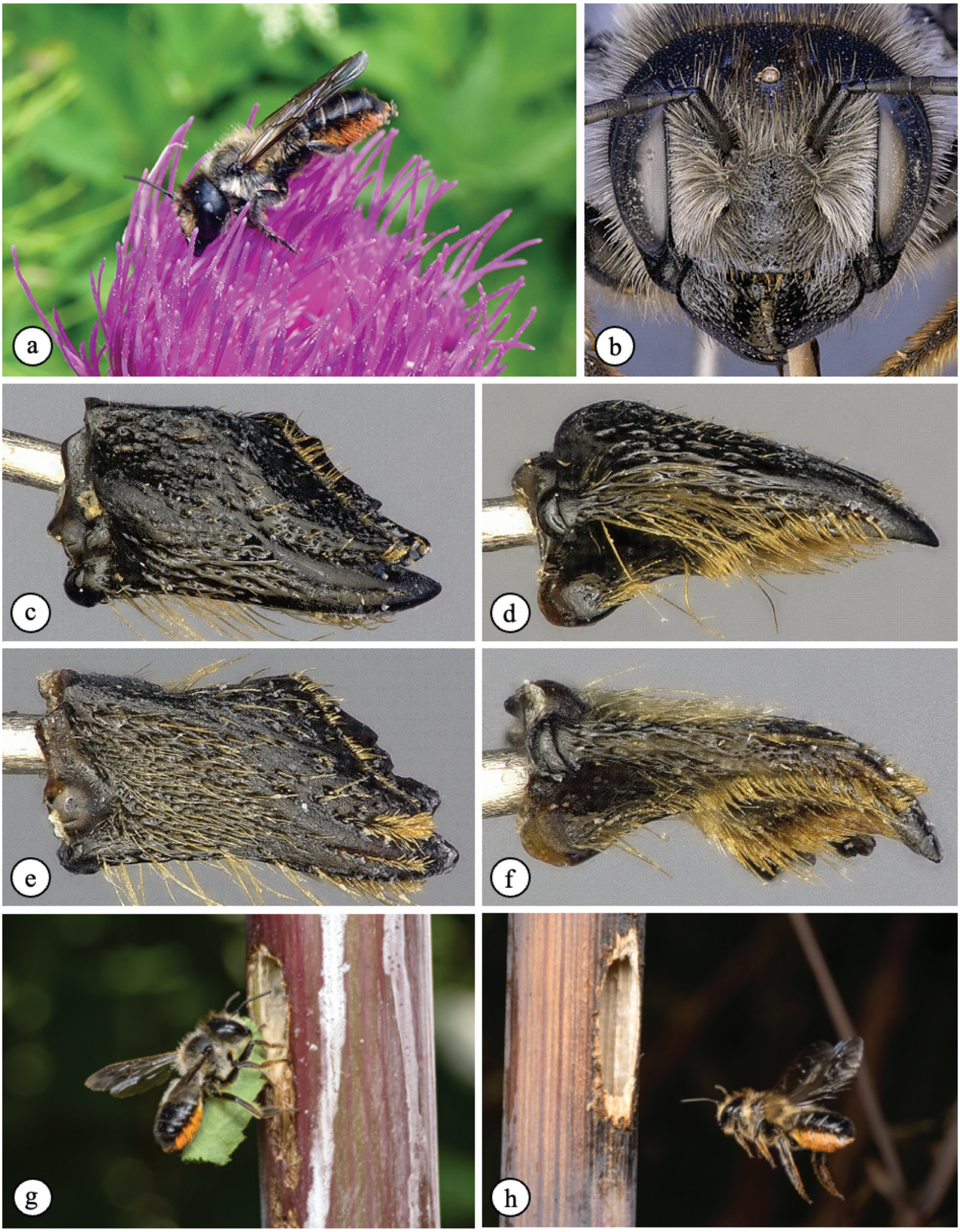

Figure 1. *Megachile genalis* and *M. centuncularis*. **a.** Female of *M. genalis* visiting *Cirsium palustre*; **b.** Head of *M. genalis* with basally inflated mandibles; **c, d.** Specialised mandible of *M. genalis* in **c.** Front view and **d.** Ventral view; **e, f.** Unspecialised mandible of *M. centuncularis* in **e.** Front view and **f.** Ventral view; **g, h.** Female of *M. genalis* entering her nest with: **g.** A rosaceous leaf fragment and **h.** Asteraceae pollen in the metasomal scopa.

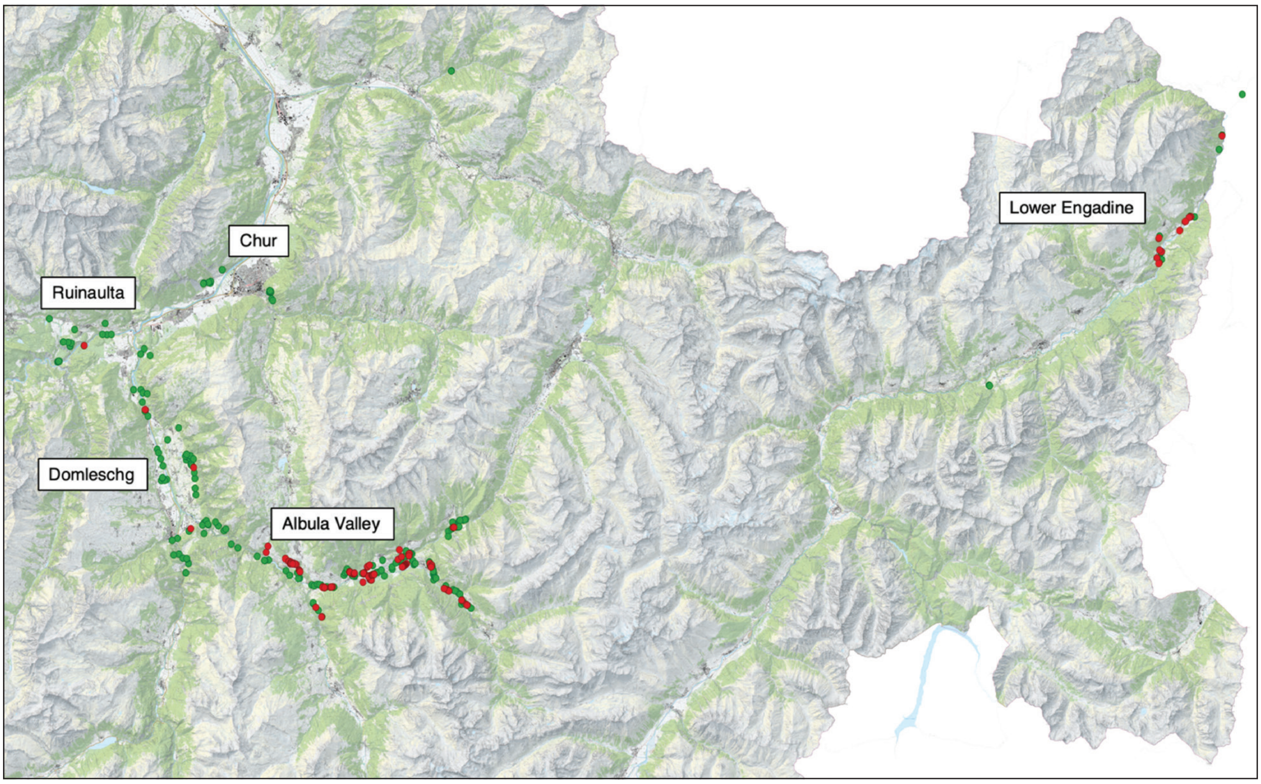

Figure 2. Distribution of *Megachile genalis* and *Peucedanum verticillare* in the eastern Swiss Alps. Red dots = nests of *M. genalis* discovered in 2023, green dots = records of *P. verticillare* after 1999.

For nests discovered in living plant stems, the following parameters were recorded in the field: i) plant species, ii) distance between ground and lower margin of nest opening, iii) longitudinal and transverse diameter of nest opening, iv) outer diameter of stem at level of nest opening, and v) compass direction of nest opening. In contrast to nests in living stems, which were left undisturbed in place with few exceptions, nests in dead and thus at least one year old stems were collected to record the following additional parameters in the laboratory: vi) inner diameter of stem at level of nest opening, vii) presence and composition of (traces of) nest plugs, viii) position and number of brood cells, ix) outer and inner diameter of stem at level of brood cells, and x) larval mortality and brood parasites. The identification of brood parasites was based on dead specimens found inside the brood cells. As all nests discovered in dead stems turned out be old nests from the last year or the year before the last year (see Results), the parameters vi)–x) could not be assessed for each old nest resulting in different parameter sample sizes.

To identify the plant sources of the leaf fragments used by the females of *M. genalis* to construct the brood cells, the outer leaf layers were removed from 157 cells of 20 nests collected in eight different subplots in the Albula Valley. The leaf fragments from all cells of a nest were pooled resulting in 20 leaf samples. These samples were analysed by AIM - Advanced Identification Methods GmbH (Leipzig, Germany) applying DNA metabarcoding. After homogenisation of the leaf fragments, DNA of 80 mg leaf material from each nest was extracted with the Qiagen DNeasy Plant Kit following the manufacturer's manual. From the extracted DNA, barcoding sequences of the nuclear marker ITS2 were PCR amplified using target specific next-generation sequencing primers and analysed by amplicon sequencing on the Illumina MiSeq platform. Amplified sequences with a minimum length of 100 base pairs were kept and clustered into a total of 192 operational taxonomic unit sequences (OTU). Given the many pitfalls of the metabarcoding approach (Förster et al. 2023), the automated identifications to genus and species level were manually checked by entering all OTU sequences represented by more than five reads (n = 62) into the BOLD identification system for ITS (https://boldsystems.org/index.php/IDS_OpenIdEngine; accessed January 2024). For each OTU sequence, the plant taxon with the highest score ("Best ID") was selected. If the Best ID referred to a species that is either not native to Central Europe or does not occur in the study area, the identification was left at genus level. Overall, the automated identifications of 53% of all OTU sequences had to be adjusted. The determination of the proportion of plant taxa used for brood cell construction was limited to OTU sequences with more than five reads, which accounted for 99.8% of the total number of reads.

To assess whether the unique shape of the female mandibles of *M. genalis* might be related to the species' habit to gnaw holes into the walls of plant stems, the mandibles of the following European *Megachile* species were compared with those of *M. genalis* based on photomicrographs of three amputated and unworn mandibles per species: *M. alpicola* Alfken, *M. centuncularis* (L.), *M. lapponica* Thomson, *M. ligniseca* (Kirby), *M. melanopyga* Costa, *M. octosignata* Nylander, *M. pyrenaea* Pérez, *M. pilicrus* Morawitz, and *M. versicolor* Smith. These species all belong to the same subgenus *M.* (*Megachile*) as *M. genalis* and usually nest in preexisting cavities above or more rarely below ground. In contrast to *M. genalis*, they need free access to the nest cavity and do not use their mandibles to gnaw the nest entrances into the plant substrate (Praz 2017; Westrich 2018). To determine the ratio of mandibular length to mandibular width, the maximum length of the mandible (from the condyle to the apex of the apicalmost tooth) and the maximum width of the mandible at its base (from the acetabulum to the condyle) was measured.

Pollen hosts

To investigate the pollen host preferences of *Megachile genalis*, pollen samples from 65 brood cells originating from 39 old nests, which were collected in eight subplots in the Albula Valley, were microscopically analysed. The pollen samples were taken either from pollen remains in cells with dead larvae (n = 22 cells) or from the faeces layer surrounding the cocoon wall (n = 43 cells; Fig. 6f). The digested pollen grains in the larval faeces could still be identified based on the characteristics of the exine, which remained intact during the passage through the larval gut. The pollen samples from the cells and the faeces were dissolved in a drop of distilled water and embedded in glycerine gelatine on a microscopic slide. For each sample, 300 pollen grains were identified under a light microscope at a magnification of 400 × along a randomly selected line across the coverslip (12 × 12 mm). If the sample contained several pollen types, the relative pollen volume of each pollen type was determined by correcting the number of counted grains with the volume of the pollen types contained in the sample.

Results

Distribution

Nests of *Megachile genalis* were found in four of the five study areas at elevations between 600 m and 1270 m (Fig. 2). With 176 nests discovered, the Albula Valley proved to be the stronghold of *M. genalis* in the eastern Swiss Alps, followed by the Lower Engadine (23 nests), the Domleschg (5 nests) and the Ruinaulta (1 nest). No nests were found in the surroundings of Chur, which is most probably explained by the very low number of erect plant stems suitable for nesting.

Nesting biology

Nesting site

In total, 205 nests of *Megachile genalis* were discovered. Of these nests, 141 were in living and 64 in dead stems. None of the nests found in dead stems was built in the year of discovery, indicating that the females of *M. genalis* selected only living stems for nesting.

Although all plant stems with a minimum outer diameter of 10 mm were examined for the presence of the typical oblong-oval nest openings, nests of *M. genalis* were exclusively found in stems of *Peucedanum verticillare* (Apiaceae), a monocarpic umbellifer up to 2–3 m tall that grows vegetatively for two to four years, produces a large inflorescence in the third to fifth year and then dies (Fig. 3a–c). The stems of *P. verticillare* are hollow and consist of several internodia separated by thin transverse partitions of pith at the nodi (Fig. 3d, e).

The nesting habitat of *M. genalis* coincides with the growth sites of *P. verticillare*, which – due to the plant's special life cycle – tolerates neither regular mowing nor grazing and thus occurs mainly on fallow land, along road and railway embankments, on scree slopes and ruderal areas as well as on forest clearings (Fig. 4a–h).

Nest architecture

The nests of *Megachile genalis* consisted of i) the nest opening gnawed by the female, ii) the nest closure built between the nest opening and the brood cells ("nest plug"), iii) the brood cells and iv) a facultative basal plug below the brood cells.

The *nest openings* (Fig. 5a–d) were situated at a height of 22–217 cm above ground (Table 1, Fig. 7a). They were on average 8.6 mm long and 5.9 mm wide (Table 1) and oriented in all directions, but predominantly in the sector from east to south (n = 92 of 138 nests; Fig. 7b). Almost two thirds of the nest openings were situated in the uppermost fifth of the internodium with the distance between the upper margin of the nest opening and the stem nodus above it averaging 5.1 cm or 23.2% of the total length of the internodium, which measured on average 22 cm (Table 1). The average diameter of the stem at the level of the nest opening was 14.4 mm on the outside and 10.5 mm on the inside (Table 1).

The *nest plugs* strongly varied in the material used, the thickness and the position within the stem. In 13 of 22 nests with a preserved closure, the plug was constructed from densely packed particles of pith only, which originated from the inner stem wall as was evident from the gnawing marks usually just above the plug (Fig. 5f); in one case, two layers of pith particles in a distance of 9.5 cm were present. In four nests, the plug consisted of 9–10 loosely to densely packed leaf fragments (Fig. 5g). In two nests, the plug was two-layered with an upper layer of densely packed pith particles immediately adjacent to a lower layer of 8–10 leaf fragments (Fig. 5h). And in one nest each, the plug consisted of 12 leaf fragments intermixed with pith particles (Fig. 5i), of densely packed grass fibres (Fig. 5j), and of three adjacent layers built from a mixture of pith particles and grass fibres above, followed by an empty space and densely packed pith particles in the middle and several leaf fragments below (Fig. 5k). The thickness of the one- and two-layered plugs ranged from 5–25 mm (Table 1), while the single three-layered plug was 59 mm thick including the 15 mm long empty space in between. The plugs of 31 nests were positioned 1.5–45.0 cm below the nest opening (Table 1), either

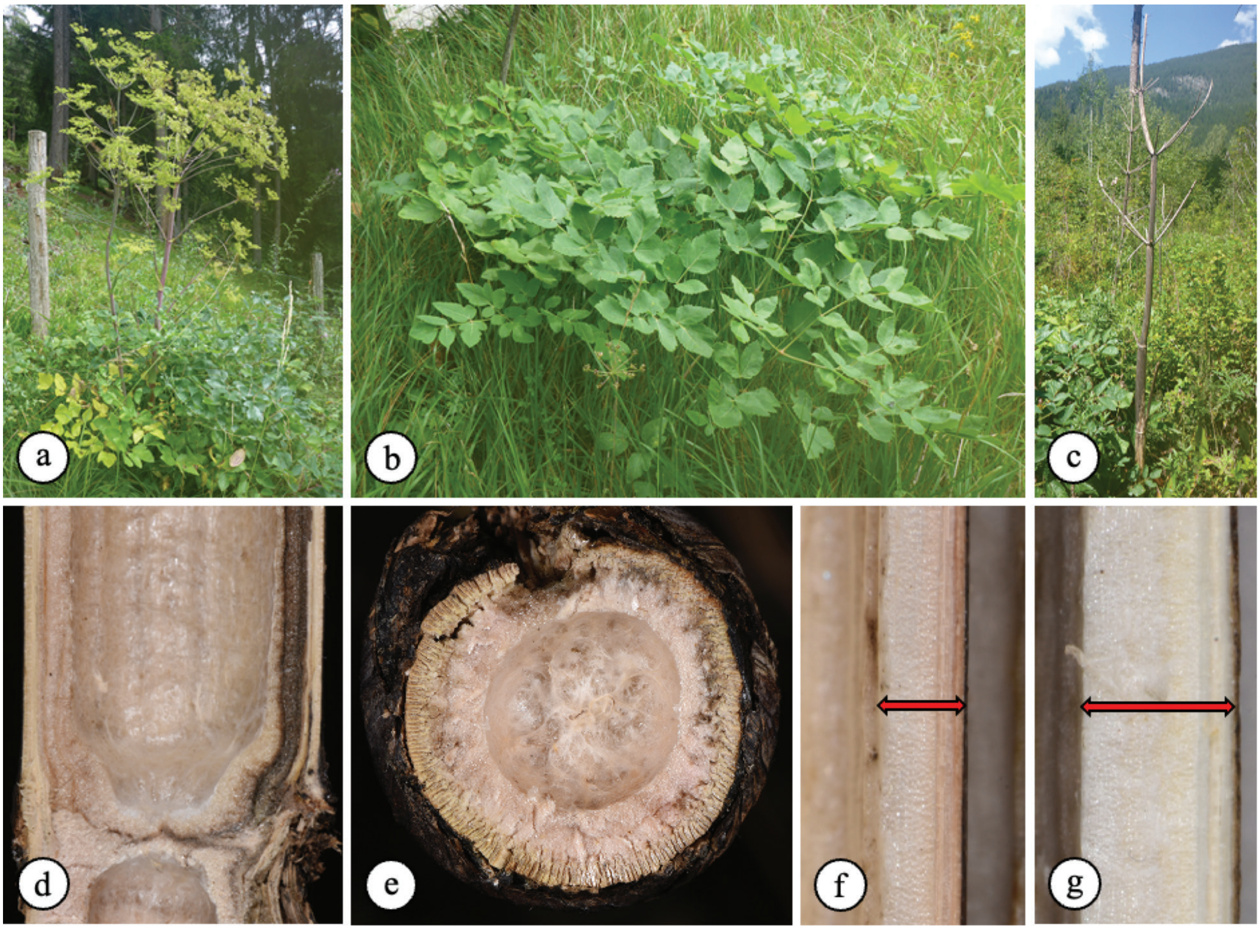

Figure 3. *Peucedanum verticillare*. **a.** Plant in flower; **b.** Leaf rosette; **c.** Dead plant; **d, e.** Transverse partition of pith between two internodia **d.** In lateral view and **e.** In top view; **f, g.** Thickness of stem wall at level of **f.** Nest opening (1.8 mm) and **g.** Brood cells (3.2 mm).

within the same internodium as the nest opening (n = 8), within the same internodium as the brood cells (n = 14), within the same internodium as both nest opening and brood cells (n = 6) or within an internodium between the nest opening and the brood cells (n = 3). They were built either in the central section of the internodium (n = 24) or directly above or below the nodus (n = 7).

The *brood cells* were mostly located far below the nest entrance; the distance between the bottom of the lowest brood cell and the nest opening was on average 49.9 cm (Table 1, Fig. 7d). In 9 (16.1%) of 56 nests, the brood cells were placed in the same internodium as the nest opening, in 17 nests (30.4%) one internodium below, in 20 (35.7%) two internodia below, in 9 (16.1%) three internodia below and in 1 nest (1.7%) four internodia below. As the internodia are separated from each other by 2–4 mm thick transverse partitions consisting of soft pith (Fig. 3d, e), female bees that did not construct the brood cells in the same internodium as the nest opening had to gnaw through one or more partitions (Fig. 5e). The average diameter of the stem at the level of the brood cells was 20.0 mm on the outside and 14.3 mm on the inside and thus about 5 mm wider than at the level of the nest opening (see above and Table 1).

The nests contained 1–14 brood cells (Fig. 7c). In 41 of 49 nests, the cells were in one contiguous, cigar-like series (Fig. 6a) either at the bottom of the internodium directly adjacent to the nodus (n = 28) or in the central section of the internodium (n = 13); in the other nests, the cells were divided into two (n = 6) or three series (n = 2; Fig. 6b) distributed in one (n = 2) or two internodia (n = 6). As an exception, two of 56 nests contained one and four downwards-facing brood cells in the internodium above the nest opening in addition to the brood cells below the nest opening, and one stem contained two nests, each with its own opening.

The walls of the brood cells consisted of several layers of loosely connected and easily detachable leaf fragments on the outside and a few layers of tightly connected leaf fragments on the inside (Fig. 6e, h, i). While the outer leaf layer varied in thickness and in the number of leaf fragments depending on the inner diameter of the stem, thus allowing the utilisation of stems ranging from 10–20 mm in inner diameter (see Table 1), the inner leaf layer was of more or less constant thickness. The width of the cells after detachment of the outer layer of leaf fragments averaged 7.9 mm, the length 13.5 mm (Table 1). By adding to the minimum inner cell width of 7 mm a few millimetres for a thin outer leaf layer, which was present in all cells, the minimum inner stem diameter required for nesting is about 10 mm. This estimate corresponds to the smallest recorded inner diameter of a stem occupied by *M. genalis* (see Table 1). By considering the thickness of the stem wall, which measured 2–4 mm at the level of the brood

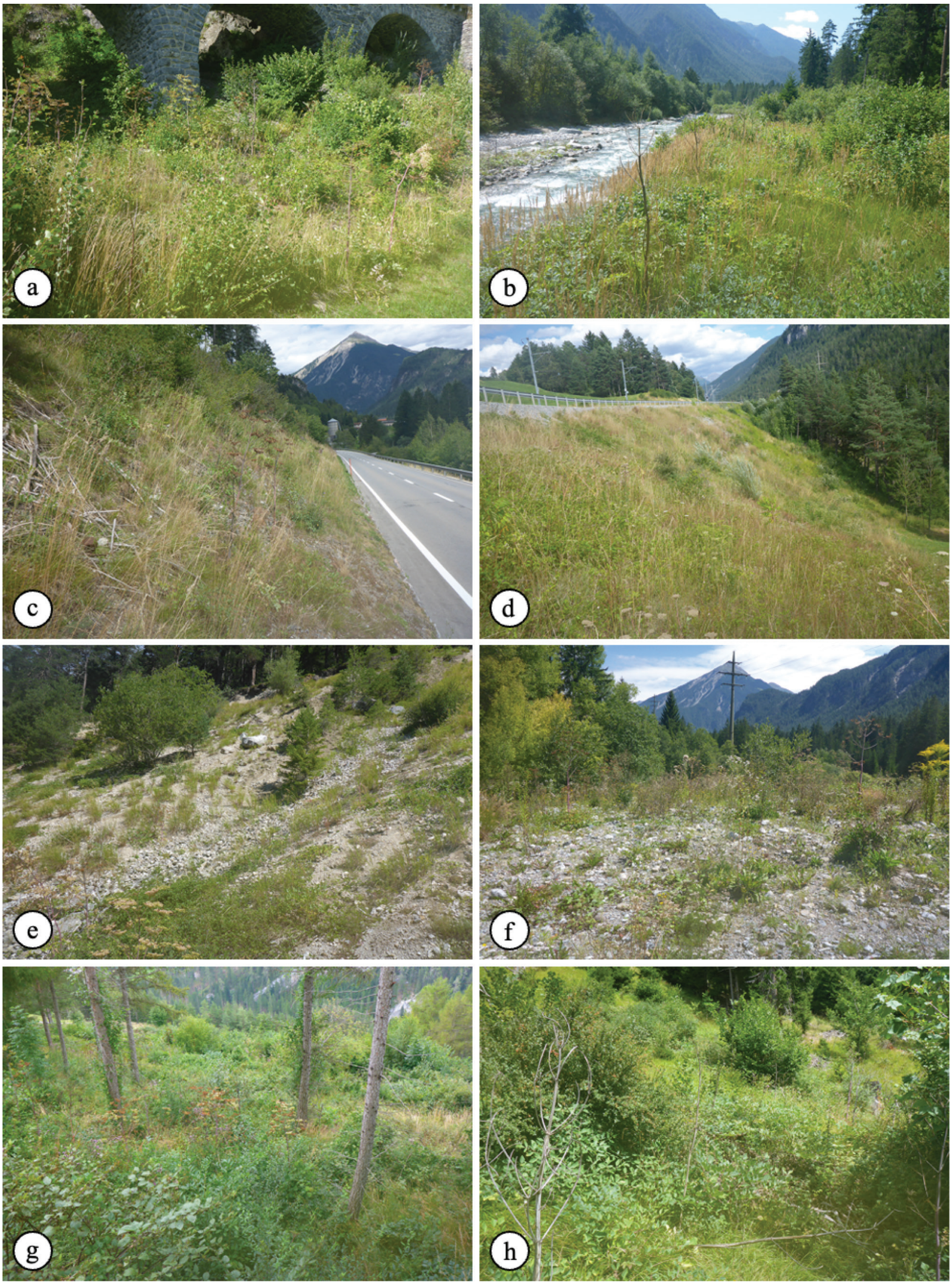

Figure 4. Nesting habitats of *Megachile genalis*. **a, b.** Fallow land; **c, d.** Road and railway embankment; **e, f.** Scree slope and ruderal site; **g, h.** Forest clearings.

cells, *M. genalis* is dependent on plant stems with an outer diameter of at least 14 mm for its reproduction, which corresponds well to the minimum outer stem diameter of 13.5 mm determined by Ruhnke (1998, 2000) for 36 nests and of 15 mm found in the present study for 53 nests (Table 1).

Basal plugs were found in 24 nests, whereas they were absent in 14 nests. The basal plug was always constructed within the same internodium as the brood cells, either directly at the nodus (n = 20) or at some distance above the nodus (n = 4). It consisted of a single layer of densely packed pith particles (n = 16; Fig. 5l), of an upper layer of leaf fragments immediately followed by a lower layer of densely packed pith particles (n = 4; Fig. 5m) or of 11–36 loosely to densely arranged leaf fragments (n = 4). The

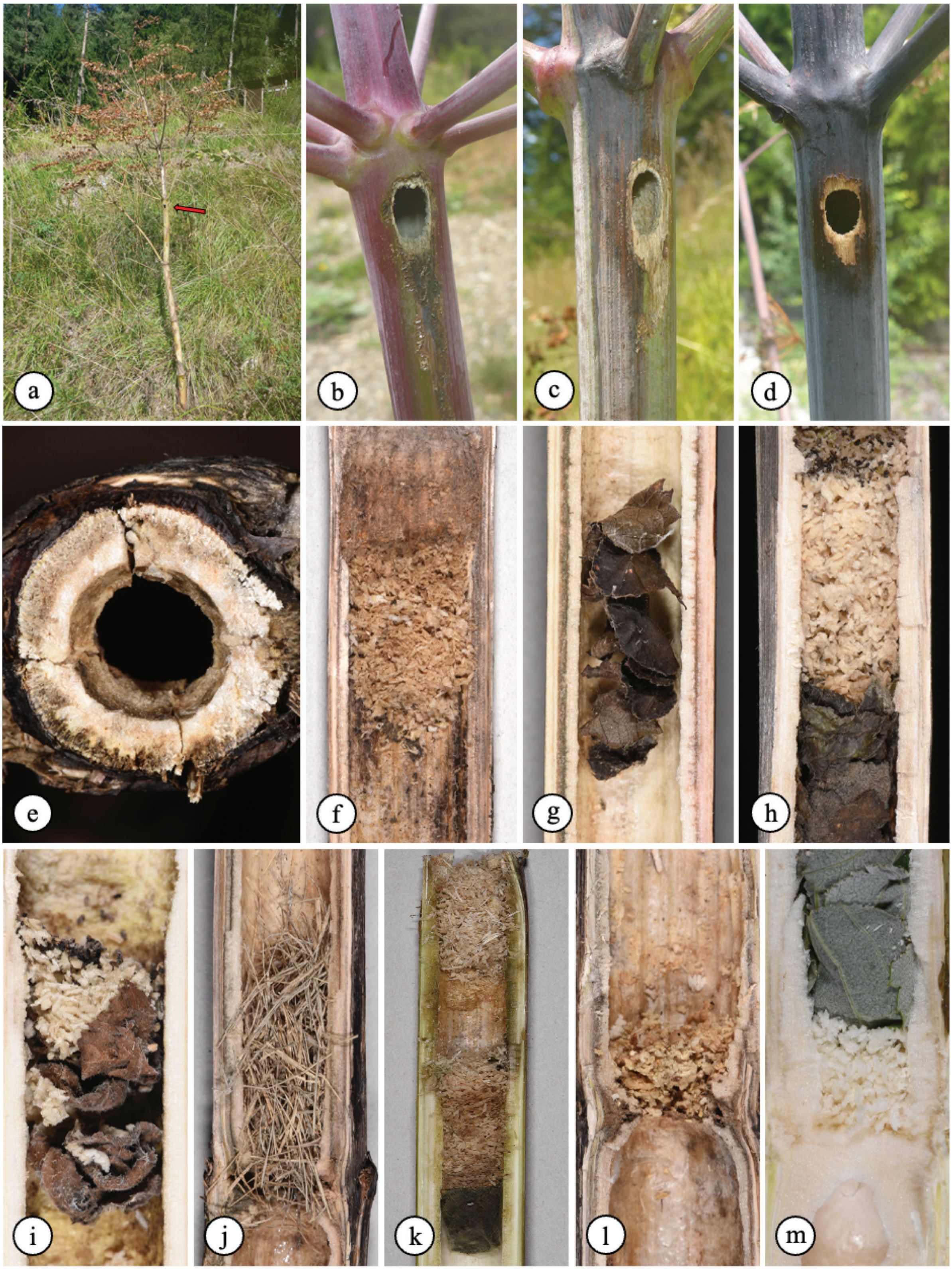

Figure 5. Nests of *Megachile genalis* in hollow stems of *Peucedanum verticillare*. **a–d.** Nest openings gnawed into the plant stem; **e.** Gnawed through transverse partition between two internodia; **f–k.** Nest plugs (for details see text); **l, m.** Basal plugs (for details see text).

thickness of the basal plugs ranged from 4–40 mm (Table 1). In the majority of nests, a basal plug was present although the transverse stem partition at the nodus was intact, suggesting that plug construction is independent of the integrity of the stem partition.

Cell-building material

The genetic analysis of the outer leaf layers of 157 brood cells from 20 nests showed that the females of *Megachile genalis* collected the leaf fragments for the construction

Figure 6. Nests of *Megachile genalis* in hollow stems of *Peucedanum verticillare*. **a.** Contiguous brood cell series; **b.** Interrupted brood cell series; **c.** Close-up of brood cells; **d.** Enigmatic marks on inside of stem wall; **e.** Brood cell with outer leaf layer removed; **f.** Layer of faeces surrounding cocoon wall; **g.** Overwintering prepupa; **h, i.** Brood cell series with outer leaf layer **h.** Present and **i.** Removed.

of the brood cells exclusively on representatives of the Rosaceae and the Betulaceae (Fig. 8). Among the Rosaceae, species of *Rosa* (e.g. *R. canina* L.) and *Rubus* (e.g. *R. caesius* L., *R. idaeus* L.) as well as *Prunus avium* L., *Fragaria vesca* L. and *Sorbus aucuparia* L. served as sources for the leaf fragments. Among the Betulaceae, *Corylus avellana* L. was used. All these species are shrubs or trees except for the herbaceous *F. vesca*. Although metabarcoding does not allow exact quantification, the percentages of OTU sequences obtained indicate that

Table 1. Parameters of nests of *Megachile genalis* constructed in hollow stems of *Peucedanum verticillare* (Apiaceae) in the eastern Swiss Alps.

Parameter	Mean ± SD	Range	n
Height of nest opening above ground	110 ± 37 cm	22–217 cm	191
Length of nest opening	8.6 ± 1.3 mm	5.5–12 mm	149
Width of nest opening	5.9 ± 0.6 mm	4.5–7.5 mm	149
Outer stem diameter at nest opening	14.4 ± 2.9 mm	9–28 mm	185
Inner stem diameter at nest opening	10.5 ± 2.7 mm	6.5–17 mm	63
Distance between upper margin of nest opening and stem nodus above it	5.1 ± 5.1 cm	0.1–17 cm	59
Distance between upper margin of nest opening and stem nodus above it relative to total length of internodium	23.2 ± 22.7%	0.4–87.9%	59
Thickness of nest plug	16.4 ± 6.7 mm	5–25 mm	17
Distance between centre of nest plug and nest opening	19.7 ± 11.5 cm	1.5–45 cm	31
Distance between bottom of lowest brood cell and nest opening	49.9 ± 21.7 cm	10.5–99 cm	56
Outer stem diameter at brood cell level	20.0 ± 3.2 mm	15–27 mm	53
Inner stem diameter at brood cell level	14.3 ± 2.5 mm	10–20 mm	51
Number of brood cells	6.1 ± 3.7	1–14	54
Length of brood cell after detachment of outer leaf fragment layer	13.5 ± 1.0 mm	11–16 mm	68
Width of brood cell after detachment of outer leaf fragment layer	7.9 ± 0.7 mm	7–9 mm	68
Thickness of basal plug	13 ± 12.0 mm	4–40 mm	10

R. canina, *R. idaeus* and *R. caesius* were the most important leaf fragment sources (85.2% of the total number of reads), but that other representatives of the Rosaceae as well as *C. avellana* were also occasionally exploited (all below 6.5% of reads). The great importance of the Rosaceae is also shown by the finding that their leaves were recorded in all 20 nests analysed (Table 2), whereas leaves of *C. avellana* were only found in six nests with more than 1% reads. However, with 97% reads, *C. avellana* was the predominant leaf fragment source in one nest (Fig. 8).

Brood mortality and parasites

In a total of 284 brood cells examined, *Megachile genalis* did not reach the imaginal stage in 159 cells (56%) due to parasitation, mould or unknown reasons. This figure is a minimum estimate as it was not possible to determine whether *M. genalis* or a similarly sized brood parasite had emerged from brood cells with normally sized eclosion holes. In 21 (46%) of 46 nests, reproduction completely failed, whereas the proportion of successful brood cells exceeded 50% in only 18 nests (Fig. 7e).

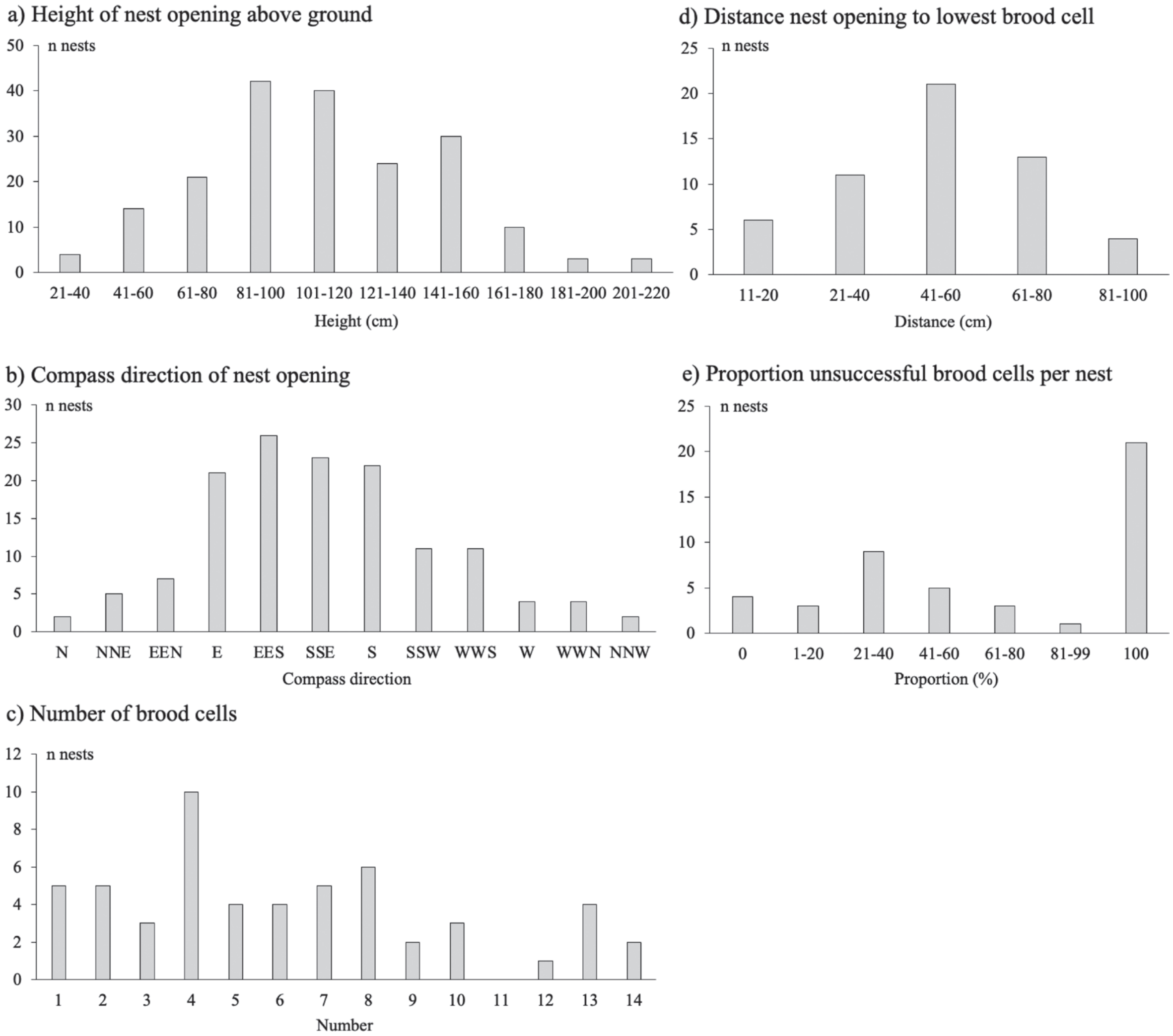

Figure 7. Nest parameters of *Megachile genalis*. **a.** Height of nest opening above ground (n = 191 nests); **b.** Compass direction of nest opening (n = 138 nests); **c.** Number of brood cells per nest (n = 54 nests); **d.** Distance between nest opening and bottom of the lowest brood cell (n = 55 nests); **e.** Proportion of unsuccessful brood cells per nest (n = 46 nests).

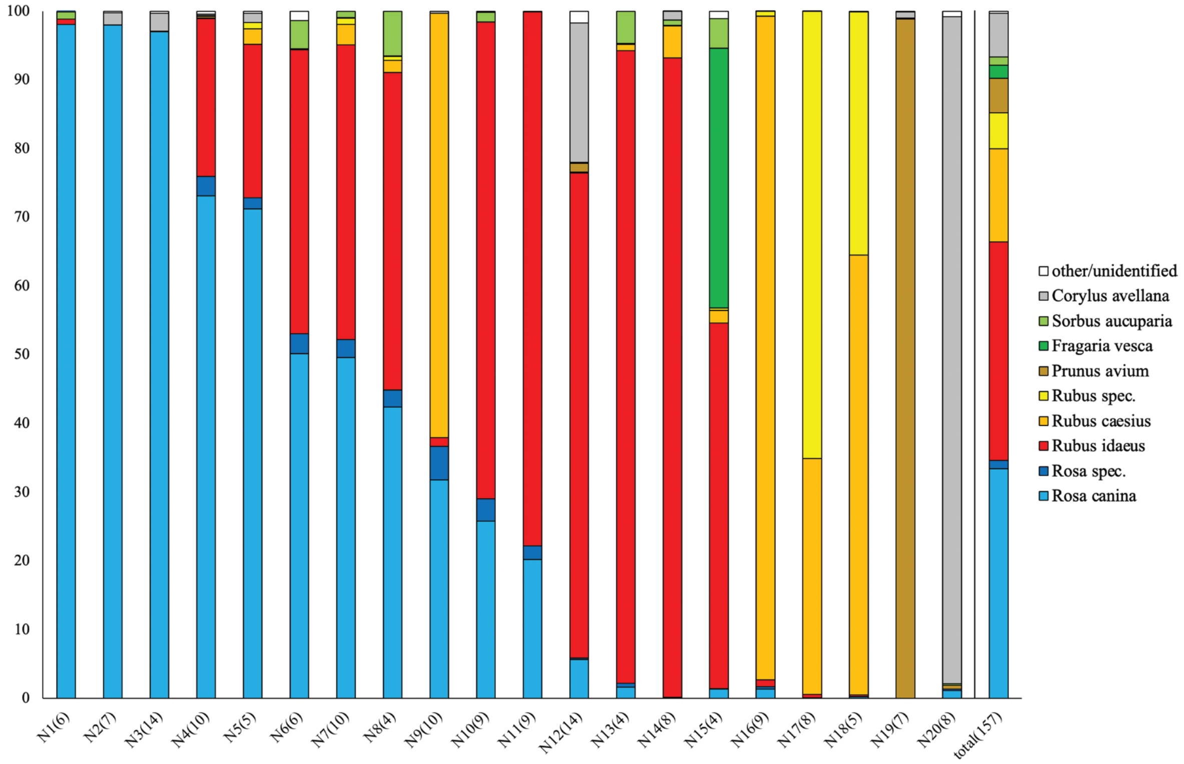

Figure 8. Origin of the leaf fragments used by *Megachile genalis* to build the brood cells in 20 nests collected in eight subplots in the Albula Valley (Grisons, Switzerland). For each nest, the percentage of ITS2 sequence reads for the identified plant taxa is given.

Table 2. Number and percentage of nests of *Megachile genalis* containing leaf fragments of the listed plant taxa based on DNA metabarcoding. Only plant taxa with more than 1% ITS2 sequence reads in a nest were considered. n = 20 nests collected in eight subplots in the Albula Valley (Grisons, Switzerland).

Plant taxon	n	%
Rosaceae	20	100
Rosa	16	80
Rosa canina	16	80
Rosa spec.	8	40
Rubus	15	75
Rubus idaeus	12	60
Rubus caesius	9	45
Rubus spec.	2	10
Sorbus aucuparia	6	30
Prunus avium	2	10
Fragaria vesca	1	5
Betulaceae	6	30
Corylus avellana	6	30

The most important brood parasite was *Melittobia acasta* (Walker) (Hymenoptera, Eulophidae), which had infested 53 (19%) of 284 brood cells and 13 (28%) of 46 nests, followed by *Exeristes roborator* (Fabricius) (Hymenoptera, Ichneumonidae; n = 4/2), *Xylophrurus augustus* (Dalman) (Hymenoptera, Ichneumonidae; n = 2/2), *Coelioxys inermis* (Kirby) (Hymenoptera, Megachilidae; n = 1/1), *Trichodes apiarius* L. (Coleoptera, Cleridae; n = 1/1) and an unidentified bombyliid fly (Diptera, Bombyliidae; n = 1/1). Furthermore, a female most probably of *Lasiambia* spec. (Diptera, Chloropidae) was observed entering a nest of *M. genalis*, adding a further species to the list of the bee's brood parasites in the study area. For the vast majority of the unsuccessful brood cells, the cause of mortality could not be determined.

Female mandibular shape

The comparison of the female mandible of *Megachile genalis* (Fig. 1c, d) with that of closely related *Megachile* species of the same subgenus, such as *M. centuncularis* (Fig. 1e, f), revealed three differences. First, the outer surface of the mandible of *M. genalis* is strongly inflated at its base, whereas the basal outer surface of the mandible of the other *M.* (*Megachile*) species is plane; second, the mandible of *M. genalis* is shorter relative to its basal width compared to the other species (about 1.8 × as long as wide in *M. genalis* compared to about 2.0 × as long as wide in e.g. *M. centuncularis*); and third, the mandible of *M. genalis* is apically barely curved inwards in contrast to the related species, which possess mandibles that are distinctly curved inwards.

Pollen hosts

The microscopic analysis of pollen remains and larval faeces from 65 brood cells and 39 nests revealed that more than 99% of all pollen collected by *Megachile genalis* originated from flowers of the Asteraceae (Table 3). Within the Asteraceae, representatives of all three subfamilies (Asteroideae, Carduoideae, Cichorioideae) were exploited. However, species of the Carduoideae were by far the most important pollen sources and their pollen was recorded in every brood cell, suggesting that *M. genalis* has a clear preference for this subfamily and particularly for thistles, whose pollen was represented by significantly higher proportions in the pollen samples than that of *Centaurea*.

Table 3. Pollen host spectrum of *Megachile genalis* in the eastern Swiss Alps. n = 65 brood cells from 39 nests collected in eight subplots in the Albula Valley (Grisons, Switzerland).

Pollen host	% pollen grain volume	Number of brood cells with pollen type (%)
Asteraceae	99.1	65 (100)
Carduoideae	84.4	65 (100)
Thistles (*Arctium, Carlina, Cirsium, Onopordum*)	72.2	62 (95.4)
Knapweeds (*Centaurea*)	12.2	40 (61.5)
Cichorioideae	13.4	45 (69.2)
Asteroideae	1.2	10 (15.4)
Other taxa	0.9	28 (43.1)

Discussion

The present study revealed that *Megachile genalis* is highly specialised in the eastern Swiss Alps with respect to nesting site, cell-building material and pollen hosts. The species nested exclusively in living stems of *Peucedanum verticillare* (Apiaceae) in the investigated plots, it used mainly leaf fragments from shrubs and trees of the Rosaceae for brood cell construction and it exploited only flowers of the Asteraceae for pollen.

Distribution

The occurrence of *Megachile genalis* in the eastern Swiss Alps is closely linked to larger stands of *Peucedanum verticillare*, and the distribution of the plant and the bee species largely coincides in the Grisons (Fig. 2). Since *P. verticillare* grows not only in man-made habitats such as fallow land or road and railway embankments, but typically also in disturbed natural habitats such as scree slopes, forest clearings or floodplains, the occurrence of *M. genalis* in the eastern Swiss Alps may have existed already before humans began to open up the forest-dominated landscape in the Neolithic period (ca. 5500-2200 years BC, Hitz et al. 2023).

Nesting biology

Nesting site

In the study area, nests of *Megachile genalis* were invariably discovered in living plant stems. This observation is in line with the findings of other authors (Ruhnke 1998, 2000; Westrich 2018; Wiesbauer 2023) and shows that *M. genalis* is an exception among the stem-nesting bees of Europe in that it builds its brood cells in living rather than dead stems. To the best of our knowledge, the only other bee species known to use living stems as nesting site is the Nearctic *Megachile montivaga* Cresson, which belongs to the same subgenus as *M. genalis* and was found to nest in green stems of thistles (Orr et al. 2015). However, in contrast to *M. genalis*, which appears to be strictly specialised to erect plant stems, *M. montivaga* has a wider nesting niche and also nests in the ground or in trap nests (Orr et al. 2015 and references therein). A possible advantage of colonising living rather than dead stems could be that living stems are more firmly anchored in the soil compared to dead stems and are therefore less likely to fall over, which would be associated with a high mortality of the progeny due to increased humidity and/or predation near the ground. Similarly, since the likelihood of being destroyed or becoming unsuitable for nesting due to bending or falling increases with the age of the stems, specialization to living stems may be advantageous in that it results in a larger number of potential nesting sites available to the bees.

Due to its body size and brood cell architecture, *M. genalis* requires thick nesting stems with an inner diameter of at least 10 mm and – depending on the thickness of the stem wall – an outer diameter of at least 13–14 mm. Apart from *Peucedanum verticillare*, there were only a few plant species in the study area that had stems with an outer diameter exceeding 12 mm. These species included *Dipsacus fullonum* L. (Caprifoliaceae), which, however, occurred only very rarely and locally in gardens, *Angelica sylvestris* L. (Apiaceae), which usually grew in (semi-) shaded and rather damp locations that were suboptimal for *M. genalis*, *Heracleum sphondylium* L. (Apiaceae), which – as a typical species of nutrient-rich meadows – was almost always mown before the flight period of *M. genalis* had started, as well as species of *Verbascum* (Scrophulariaceae), *Arctium*, *Carduus* and *Cirsium* (Asteraceae), all of which have pithy stems and are probably colonised only in the absence of hollow stems. The finding that *M. genalis* exclusively nested in stems of *P. verticillare* in the study area may therefore be explained less by a local specialisation than by the lack of alternatives. In fact, *M. genalis* appears to be rather flexible in the selection of the plant species used as nesting substrate: its nests were found in thick stems of Apiaceae (*Angelica*, *Conium*, *Heracleum*), *Dipsacus* (Caprifoliaceae), *Allium* (Amaryllidaceae), Asteraceae (*Carduus*, *Cichorium*, *Cirsium*, *Dahlia*, *Echinops*) and in an exceptional case in the leaf sheath of *Zea mays* (Poaceae) (Buysson 1902; Benoist 1940; Grandi 1954, 1957; Ruhnke 1998, 2000 and references therein; Hausl-Hofstätter 2007; Westrich 2018; Sobieraj-Betlińska 2021; Wiesbauer 2023). Interestingly, in eastern Austria where *M. genalis* has two generations per year due to the warm climate, the females of the first generation preferentially nest in soft and thin-walled stems of *Allium* (Amaryllidaceae), which are easy to penetrate, whereas those of the second generation colonise hard and thick-walled stems of *Cichorium intybus* (Asteraceae) and various species of Apiaceae, which are more likely to persist over the winter (Wiesbauer 2023).

Nest architecture

The examination of numerous, mainly one- to two-year-old nests of *Megachile genalis* in stems of *Peucedanum verticillare* revealed a striking variability with respect to the characteristics of the nest opening (size, height above ground, orientation, position within internodium), the nest plug (thickness, material, position both in stem and within internodium), the brood cells (number, linear or interrupted series, position both in stem and within internodium), and the basal plug (presence or absence, thickness, material, position within internodium). Particularly intriguing was the use of different materials either alone or in combination for constructing the nest plug, including particles of pith, leaf fragments and grass fibres. This high species-specific flexibility is also apparent in the population of *M. genalis* studied by Ruhnke (1998, 2000), where the females colonized (and hollowed out) pithy rather than hollow stems and where about two thirds of all nests contained brood cells above and below the nest opening, which was observed only in two nests in the present study.

Although this high variability makes it difficult to capture the nest architecture of *M. genalis* at a glance, the "average nest" in the eastern Swiss Alps can be described as consisting of i) a nest opening that is 9 mm long and 6 mm wide, is located one meter above the ground, is oriented towards southeast and is gnawed in the uppermost fourth of the internodium, ii) a nest plug that is 15 mm thick, consists of densely packed particles of pith and lies 20 cm below the nest opening in the central section of the same internodium as the brood cells, iii) six brood cells that are constructed in one contiguous series in the second internodium below the nest opening and are located at the bottom of the internodium in a distance of 50 cm from the nest entrance, and iv) a basal plug that is 13 mm thick, consists of densely packed pith particles and lies at the nodus of the same internodium as the brood cells.

One unexpected characteristic of the nests of *M. genalis* in the study area was the large distance between the nest opening and the bottom of the lowest brood cell, which measured on average about half a metre and maximally almost one metre. The reason for this large distance is possibly related to the thickness of the stem wall, which need to be gnawed through by the female bee to enter the stem. The stem wall thickness of *P. verticillare* increased from an average of 2 mm at the level of the nest opening to an average of 3 mm at the level of the brood cells and up to 5 mm near the stem base (Table 1, Fig. 3f, g). As the walls of living stems are very hard, the females might need less time and energy to gnaw the nest entrance in the upper thinner part of the stem, where the diameter is too small to place the brood cells, than to penetrate the lower thicker part, where the brood cells will later be placed, even if this means that one or more soft and thin transverse partitions of pith have to be gnawed through at the nodi.

In many nests, zigzag-shaped, narrow and longitudinal to transverse marks were visible in the pith of the inner walls of the internodium that contained the brood cells (Fig. 6d). These marks were most probably bitten by the females with their mandibles, but are most likely not related to the acquisition of nest-building material, e.g. for the construction of the basal plug, since the pith along these bite marks was not completely detached from the stem wall. It is equally unlikely that these marks served to roughen the walls for better attachment of the brood cells within the stem cavity, as they were also found outside the brood cell area. The function of these conspicuous bite marks therefore remains a mistery.

Cell-building material

In the study area, species of Rosaceae (*Rosa*, *Rubus*, *Prunus*, *Sorbus*, *Fragaria*) were by far the most important sources of the leaf fragments used by *Megachile genalis* to construct its brood cells. This finding is in agreement with observations made by Dudich (1884), Henschel (1888), Friese (1923) and Wiesbauer (2023), who recorded leaves of the rosaceous genera *Rosa*, *Rubus* and *Pyrus* as cell-building material of *M. genalis*. Six of the eight plant genera identified by Ruhnke (1998) as leaf sources for *M. genalis* also belonged to the Rosaceae including *Rosa*, *Rubus*, *Prunus*, *Fragaria*, *Crataegus* and *Agrimonia*. Thus, *M. genalis* seems to have a strong preference for leaves of Rosaceae to build its brood cells. However, the preference for Rosaceae leaves is not exclusive as leaves of other plant taxa, such as *Acer* (Sapindaceae), *Corylus* (Betulaceae) or *Robinia* (Fabaceae), are rarely also used (Ruhnke 1998; Schweighofer 2021; this study).

Several osmiine bee species of *Osmia* and *Hoplitis* (Megachilidae) were also found to preferentially use leaves of the Rosaceae as nest-building material (Müller 1994; Prosi et al. 2016; Müller and Richter 2018; Müller et al. 2019, 2020), suggesting that the selection of leaves for nest construction by *M. genalis* and the osmiine bees is far from accidental and that leaves of Rosaceae might possess particularly favourable properties. A special leaf consistency that might render rosaceous leaves especially suitable for cutting the fragments and manufacturing the brood cells by leafcutter bees hardly explains the high importance of this plant family for megachilid bees, since the *Osmia* and *Hoplitis* species mentioned above use to masticate the Rosaceae leaves to pulp, which does not seem to require a particular leaf consistency. One alternative, albeit very speculative explanation for the importance of Rosaceae as source for nest-building material may be that the defensive secondary compounds contained in Rosaceae leaves provide a particularly effective protection for bee larvae against mould, pathogenic bacteria or predators.

Brood mortality and parasites

The brood mortality of *Megachile genalis* in the study area amounted to 56% and in almost half of all nests reproduction completely failed. As the method applied in the present study, i.e. the examination of old nests, did not allow to determine whether *M. genalis* or a similarly sized brood parasite had emerged from brood cells with normally sized eclosion holes, both brood mortality and the proportion of unsuccessfull nests were certainly higher. Given the average number of six brood cells per nest and assuming that the females constructed only one to maximally two nests during their flight period, the reproductive output of *M. genalis* in the study area in 2023 was very low and did not exceed an average of two to five offspring per female. A slightly lower brood mortality with an average of 51% failed brood cells over a period of two years was recorded for *M. genalis* in eastern Germany (Ruhnke 1998). Compared to a recent metastudy that included publications on 147 solitary bee species and found an average brood mortality of 29% for all species and 37% for cavity-nesting species (Minckley and Danforth 2019), the proportion of unsuccessful brood cells in *M. genalis* as recorded by Ruhnke (1998) and in the present study was substantially higher. Similarly high or even higher mortality rates were found in other Palaearctic megachilid bees such as *Hoplitis princeps* (52% brood mortality), *Osmia uncinata* (77%) and *Osmia pilicornis* (83%) (Prosi et al. 2016; Ivanov and Fateryga 2018; Müller et al. 2020), suggesting that overall brood mortality in megachilid bees may be higher than in other bee lineages.

Melittobia acasta (Hymenoptera, Eulophidae), which had infested 19% of the brood cells and 28% of the nests, was the main brood parasite of *M. genalis* in the study area. The other five antagonists were of much less significance and had infested together a total of 3% of the brood cells and 15% of the nests (but see methodological limitation above). Similarly, *Lasiambia* spec. (Diptera, Chloropidae) was the main brood parasite in the population of *M. genalis* investigated by Ruhnke (1998) and responsible for an estimated mortality of 10–30% of all cells, whereas the other antagonists had parasitized together less than 6% of the cells. *Melittobia acasta*, *Exeristes roborator* (Hymenoptera, Ichneumonidae), *Coelioxys inermis* (Hymenoptera, Megachilidae) and *Lasiambia* spec., which were found to parasitize nests of *M. genalis* in the study area, were also recorded by Ruhnke (1998), who additionally identified *Aritranis explorator* (Tschek) and *Cratichneumon fugitivus* (Gravenhorst) (Hymenoptera, Ichneumonidae) as well as *Coelioxys mandibularis* Nylander as brood parasites.

The new finding by Ruhnke (1998) that *Coelioxys inermis* and *C. mandibularis* develop in the nests of *M. genalis* has been overlooked in the bee literature. Interestingly, *Coelioxys inermis* has been bred so far from nests of *Megachile alpicola*, *M. centuncularis*, *M. lapponica* and *M. versicolor* (Voith 1997; Amiet et al. 2004; Westrich 2018 and references therein), all of which belong to the subgenus *M.* (*Megachile*) as *M. genalis* does, suggesting that *C. inermis* may be specialized to *M.* (*Megachile*) species as hosts. In contrast, the host spectrum of *C. mandibularis* is taxonomically wider and encompasses *Megachile versicolor*, *Hoplitis* (*Anthocopa*) *villosa* (Schenck) and probably several further *Megachile* and *Hoplitis* species of the subgenera *M.* (*Eutricharaea*), *M.* (*Megachile*), *M.* (*Xanthosarus*) and *H.* (*Anthocopa*) (Voith 1997 and references therein).

Female mandibular shape

The female mandibles of *Megachile genalis* are unique among the European *M.* (*Megachile*) species with respect to three characters: i) they possess a strongly inflated base; ii) they are shorter and more compact; and iii) they are apically barely curved inwards. These three characters are probably related to the specialised habit of the females to gnaw through the hard walls of living plant stems. The inflated base likely contains strong adductor muscles, which are capable of exerting high pressure on the stem surface. And both the shorter length and the absence of an inward apical curvature gives the mandible a compact and chisel-like shape, which seems to be well suited to transmit mandibular power onto the plant substrate. Interestingly, the latter two characters were also found in females of *Osmia nigriventris* (Zetterstedt), a boreoalpine mason bee of the subgenus *O.* (*Melanosmia*) that uses its shortened and apically almost straight mandibles to gnaw nesting burrows into hard bark and wood (Müller et al. 2019); in contrast, the female mandibles of related *O.* (*Melanosmia*) species that colonise preexisting cavities for nesting are longer and apically distinctly curved inwards, which corresponds to the difference in mandibular shape between the stem-gnawing *M. genalis* and its cavity-nesting relatives. Therefore, the peculiar shape of the female mandible of *M. genalis* is interpreted here as an adaptation that has evolved for penetrating the hard walls of plant stems.

Pollen hosts

In the study area, *Megachile genalis* collected pollen exclusively on flowers of the Asteraceae and among the Asteraceae particularly on species of the Carduoideae. Pollen of thistles (*Arctium*, *Carduus*, *Cirsium*, *Onopordum*) accounted for almost 75% of the total pollen grain volume in the brood cells analysed, which is surprising as thistles occurred only locally and rarely on pastures and ruderal areas in the study area, whereas species of *Centaurea* or Cichorioideae grew abundantly almost everywhere on nutrient-poor meadows. This suggests that *M. genalis* has a distinct preference for the pollen of thistles in the eastern Swiss Alps. This preference, however, is unlikely a local phenomenon since thistle species of *Carduus* and *Cirsium* were also noted as the preferred host plants of *M. genalis* in eastern Germany and Poland (Dorn and Weber 1988; Ruhnke 1998; Celary and Wiśniowski 2002) and as flower-visiting females of *M. genalis* were repeatedly observed on flowers of *Carduus*, *Cirsium* and *Silybum* in different regions of Germany and Austria (Sieber 1933; Schweitzer 2002; Auer in Westrich 2018; Schweighofer 2021; Tischendorf 2021). In addition to thistles, *Centaurea* (Carduoideae), *Cichorium* and *Picris* (Cichorioideae) as well as *Buphthalmum* and *Inula* (Asteroideae) are other known Asteraceae pollen hosts of *M. genalis* (Ruhnke 1998; Schweighofer 2021; present study). The results of the present study in combination with anecdotal observations in the literature confirm the oligolecty of *M. genalis* on Asteraceae as was already suspected by various authors (Ruhnke 1998, Westrich 2018). *Megachile genalis* is thus best categorised as broadly oligolectic on Asteraceae with a strong preference for thistles.

Conclusions

In the eastern Swiss Alps, *Megachile genalis* relies on i) living stems of *Peucedanum verticillare* (Apiaceae) as nesting sites, ii) green leaves of mainly *Rosa* and *Rubus* (Rosaceae) as cell-building material and iii) flowers of thistles and other Asteraceae as pollen sources. While the required nest building material is ubiquitous throughout the study area, the supply of pollen hosts and particularly nesting sites is much more limited. Measures to conserve the population of *M. genalis* in the Grisons should therefore focus on the promotion of thistles and, above all, on the conservation and propagation of suitable habitats for *P. verticillare* in close vicinity to Asteraceae-rich areas. As *P. verticillare* does not tolerate regular mowing or grazing, many of its current stands are located on fallow land that is at risk of becoming completely overgrown in the near future, since its (sporadic) management is not rewarded by agroecological schemes. The more strongly advanced strict segregation between forest and cultivated land caused by the loss of such irregularly managed fallow land probably explains the rarity of *M. genalis* in the Domleschg compared to the Albula Valley and the Lower Engadine, where still many suitable transitional habitats between forest and open land exist. In the Grisons, the fate of *M. genalis* – a rare and endangered bee species throughout Europe – will depend on whether a balance can be found between the complete abandonment of use and the annual mowing or grazing of the (potential) habitats of *P. verticillare*.

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