

Comparative biology of four *Rhodanthidium* species (Hymenoptera, Megachilidae) that nest in snail shells

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Abstract

Some species of two tribes (Anthidiini and Osmiini) of the bee family Megachilidae utilize empty gastropod shells as nesting cavities. While snail-nesting Osmiini have been more frequently studied and the nesting biology of several species is well-known, much less is known about the habits of snail-nesting Anthidiini. We collected nests of four species of the genus *Rhodanthidium* (*R. septemdentatum*, *R. sticticum*, *R. siculum* and *R. infuscatum*) in the Czech Republic, Slovakia, Catalonia (Spain) and Sicily (Italy). We dissected these nests in the laboratory and documented their structure, pollen sources and nest associates. The four species usually choose large snail shells. All four species close their nests with a plug made of resin, sand and fragments of snail shells. However, nests of the four species can be distinguished based on the presence (*R. septemdentatum*, *R. sticticum*) or absence (*R. siculum*, *R. infuscatum*) of mineral and plant debris in the vestibular space, and the presence (*R. septemdentatum*, *R. infuscatum*) or absence (*R. sticticum*, *R. siculum*) of a resin partition between the vestibular space and the brood cell. *Rhodanthidium septemdentatum*, *R. sticticum* and *R. siculum* usually build a single brood cell per nest, but all *R. infuscatum* nests studied contained two or more cells. For three of the species (*R. siculum*, *R. septemdentatum* and *R. sticticum*) we confirmed overwintering in the adult stage. Contrary to *R. siculum*, *R. septemdentatum* and *R. sticticum* do not hide their nest shells and usually use shells under the stones or hidden in crevices

within stone walls. Nest associates were very infrequent. We only found two *R. sticticum* nests parasitized by the chrysidid wasp *Chrysura refulgens* and seven nests infested with pollen mites *Chaetodactylus* cf. *anthidii*. Our pollen analyses confirm that *Rhodanthidium* are polylectic but show a preference for Fabaceae by *R. sticticum*.

Keywords

Anthidiini, bees, ecology, nest structure, phenology, pollen specialization

Introduction

There are approximately known 20,000 species of bees worldwide classified into seven families (Michener 2007). Most non-parasitic species (ca. 70%) nest underground. Among bees nesting above ground, a few species, all of them in the family Megachilidae, utilize empty gastropod shells for nesting. Megachilidae comprises approximately 4,000 species classified into seven tribes and more than 70 genera (Michener 2007; Ascher and Pickering 2020). Nesting in gastropod shells has been reported in two tribes (Osmiini and Anthidiini) and five genera (*Osmia* Panzer, *Hoplitis* Klug, *Protosmia* Ducke, *Rhodanthidium* Isensee, and *Afranthidium* Michener). In addition, there is a single record of nesting in gastropod shells for *Megachile* (*Chalicodoma*) *lefebvrei* Lepelletier (tribe Megachilini), which usually builds nests in cavities in or between rocks (Müller et al. 2018).

The largest number of species nesting in snail shells are found in the Osmiini, which includes 52 species from five genera, most of which (43 species) occur in the Palaearctic biogeographic region (Müller et al. 2018). Most species are shell-nesting specialists and only occasionally use other types of cavities. However, a few species (most in the subgenus *Osmia* (*Osmia*)) typically nest in other types of cavities and only occasionally in snail shells (for review, see Müller et al. (2018)). The tribe Anthidiini displays a wide variety of nesting behaviours, including nesting underground, using various types of cavities and building exposed nests (Michener 2007; Litman et al. 2016; Westrich 2018). Nesting in shells in this tribe has been recorded in only four Palaearctic species of *Rhodanthidium* (Erbar and Leins 2017; Baldock et al. 2018; Westrich 2018; Romero et al. 2020) and two Afrotropical species of *Afranthidium* (Gess and Gess 2008, 2014).

The genus *Rhodanthidium* comprises 13 species, eight of which occur in Europe. The genus is divided into three subgenera: *Asianthidium* Popov (three species), *Meganthidium* Mavromoustakis (one species) and *Rhodanthidium* s. str. Isensee (nine species) (Michener 2007; Kasperek 2019; Kuhlmann et al. 2021). Among *Asianthidium*, the nesting biology is known only in *R. caturigense* (Giraud) which occurs in southern and central Europe, North Africa and the Middle East. This species builds nests in soil, often in large aggregations of 130–150 females. Nests of *R. caturigense* usually have 3–6 brood cells at the end of a short burrow. Cells are usually haphazardly oriented and conform to the presence of stones and roots. This species uses plant resin and plant

fibres to build nest plugs and brood cells. Each individual brood cell has two distinct layers – the inner layer of resin and the outer layer of plant fibres (e/g/ from leaves of *Verbascum*). The plug of the nest is built from resin coated with plant fibres (Pasteels 1977; Scheuchl and Willner 2016; Kasperek 2019). The nesting biology of the only *Meganthidium* species (*R. superbum* (Radoszkowski)), which is distributed in Turkey and the Middle East, remains unknown (Kasperek 2019).

The nesting biology of the subgenus *Rhodanthidium* s. str. is only partly known for five of the nine described species. Nothing is known about the nesting biology of *R. acuminatum* (Mocsáry) from Morocco, Sicily, Greece and Turkey, *R. buteum* (Warncke) from eastern Turkey, *R. exsectum* (Pasteels) from the Middle East, and *R. ordonezi* (Dusmet) from Morocco (Kasperek 2019). The nesting biology of *R. rufocinctum* (Alfken) also remains unknown; because of its close phylogenetic relationship to *R. septemdentatum*, it is expected to nest in snail shells (Kasperek 2019). The nesting biology of the other four species has only been partly described. *Rhodanthidium septemdentatum* (Latreille) has a wide distribution across southern and central Europe, North Africa and the Middle East; *R. infuscatum* (Erichson), *R. siculum* (Spinola) and *R. sticticum* (Fabricius) are only distributed in the western part of southern Europe (Portugal to Italy) and North Africa. Erbar and Leins (2017) described the nesting biology and pollen preferences of *R. siculum*. Although various aspects of the nesting biology of the three remaining species can be reconstructed based on several short notes (*R. septemdentatum*: Xambeau 1896; Friese 1911; Armbruster 1913; Grandi 1961; Gogala 1999; Grace 2010; Kasperek 2019; *R. infuscatum*: Pasteels 1977; *R. sticticum*: Schremmer 1960; Pasteels 1977; Ortiz-Sánchez 1990; Bosch et al. 1993; Romero et al. 2020), a comprehensive study of their biology has not yet been published. In particular, the range of snail species used by these species is unknown; the only relevant publication to date is that of Romero et al. (2020), who studied the use of empty gastropod shells by adults during inclement weather and at night.

In this study, we describe the nesting biology of four species of *Rhodanthidium* (*R. septemdentatum*, *R. sticticum*, *R. siculum* and *R. infuscatum*), including the range of snail shells used, the manipulation of shells by females during nesting, the structure of the nest, the main pollen sources collected by nesting females for their brood, the overwintering stage and the nest parasites.

Methods

We collected snail shells containing nests of *Rhodanthidium* in the Czech Republic (two sites in 2017 and 2018), Slovakia (one site), Catalonia (northeastern Spain; various sites in the provinces of Barcelona, Girona and Lleida in 1996, 1999, 2001, and 2018–2021), and Sicily (Italy, one site in 2018) (Suppl. material 1: Table S1, Suppl. material 2: Table S2). A description of all the sites surveyed is provided in Appendix 1.

We dissected the snail shells in the laboratory using thick tweezers, carefully breaking off small fragments of the upper part of the shell from the aperture to the apex.

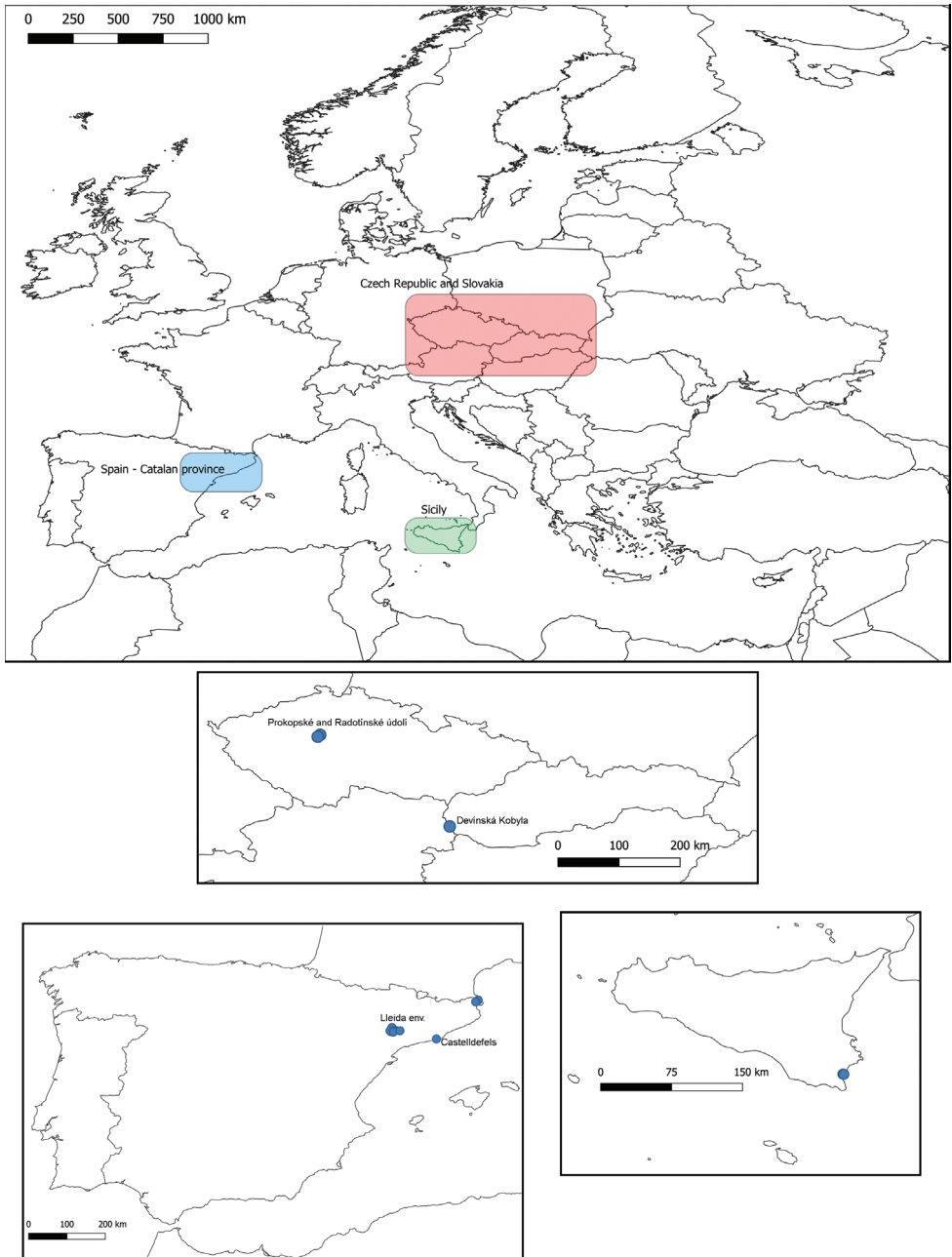


Figure 1. Location of study sites (blue dots) in the three regions surveyed.

We described the structure of the nest, including the number of brood cells and the materials used to make the plug and cell partitions, as well as any loose filling material found in the vestibular cell. We also recorded the developmental stage of the brood.

Larvae (with their food provision) and pupae of nests collected in spring/summer were transferred to microtubes closed with cotton wad and kept under laboratory conditions (20–22 °C, ca. 60% relative humidity). In September, cocoons were dissected to check the developmental stage. Adults were identified, and their sex determined.

We described the structure of the nests, took photos of some of them and made schematic drawings of the structure of nests for the four species. Photos of nests and their contents were taken using a Canon E550d digital camera with a macro lens. Final figures were created from multiple photos stacked by ZERENE STACKER software using the D-Map/P-Max algorithm. The drawings of nests were made by a pen and retouched and coloured in ADOBE PHOTOSHOP.

We took pollen samples of five nests of *R. septemdentatum*, one nest of *R. siculum* and nine nests of *R. sticticum*. Pollen samples were prepared using a standard acetolysis method (Moore et al. 1991) based on 5 min of boiling in an acetolysis mixture of sulfuric acid (H₂SO₄) and acetic anhydride (CH₃CO)₂O at a ratio of 1:9. The sample was then transferred into a mixture of water and glycerol. Slides were observed at 400× magnification using a light microscope. Pollen grains were identified using pollen atlases (Punt and Clarke 1984; Moore et al. 1991; Reille 1992; Beug 2004) and the reference collection of the Department of Botany at Charles University. An overview of the samples and types of pollen found is shown in Suppl. material 3: Table S3.

In our study of the nesting biology of *R. septemdentatum*, we attempted to determine whether females search for nesting snail shells under stones or if they transport snail shells under stones themselves. In 2018, we performed a manipulative experiment with snail shells in the locality Prokopské údolí. Based on our knowledge of the nesting sites of this species from 2017, we placed 16 empty snail shells of *Caucasotachea vindobonensis* (Férussac) on the ground surface around each of four nesting sites: four shells at a distance of up to 50 cm from the centre of the nesting site (marked with a number of the nesting site and letter A), another four shells up to 1 m (B), another four shells up to 2 m (C), and the last four shells up to 4 m (D). The snail shells were placed on 30th April 2018 (before the nesting season) and collected on 29th June (at the end or after the end of the nesting season).

Results

Rhodanthidium septemdentatum (Latreille)

Material examined. 23 nests from five localities in the Czech Republic, Slovakia and Spain (Suppl. material 2: Table S2).

Nest structure. All nests had a subterminal closing plug, a vestibular cell and one or two brood cells (Fig. 2). The vestibular cell was delimited by the closing plug and an inner partition, both made of resin and loosely filled with mineral fragments, soil and plant matter. In nests with two brood cells, there was no partition between the two (Fig. 2B). Nests with two cells appeared to be more frequent in central Europe

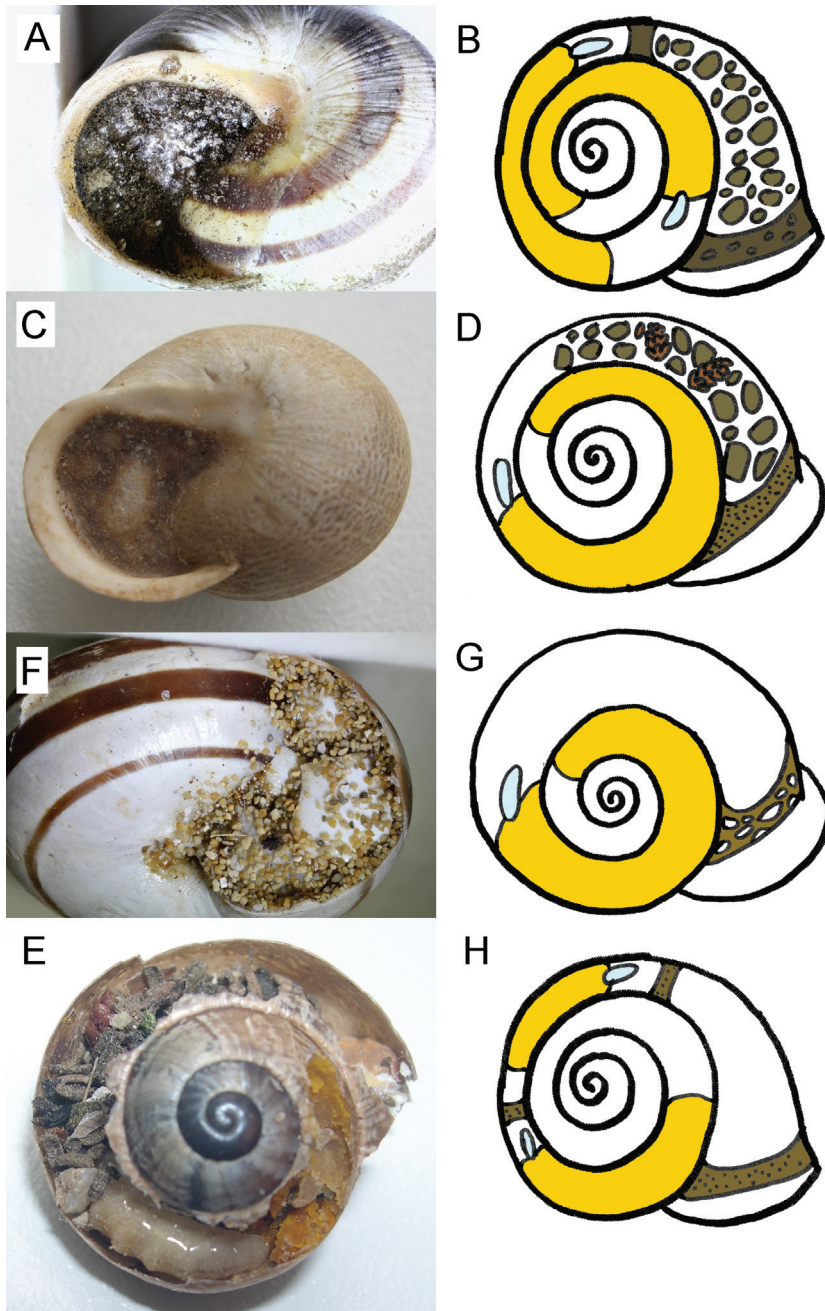


Figure 2. Photos and schematic drawings of nests of four species of *Rhodanthidium*. *Rhodanthidium septemdentatum* **A** shell of *Caucosatachea vindobonensis* with closing plug made of resin **B** schematic drawing of the inner nest structure in the shell. *Rhodanthidium sticticum* **C** shell of *Eobania vermiculata* with closing plug made of resin and soil particles **D** schematic drawing of the inner nest structure in the shell **E** photo of the shell with larva, pollen and filling of stones and plant partitions. *Rhodanthidium siculum* **F** shell of *Eobania vermiculata* with closing plug made of resin, sand and shell particles **G** schematic drawing of the inner nest structure in the shell. *Rhodanthidium infuscatum* **H** schematic drawing of the inner nest structure in the shell.

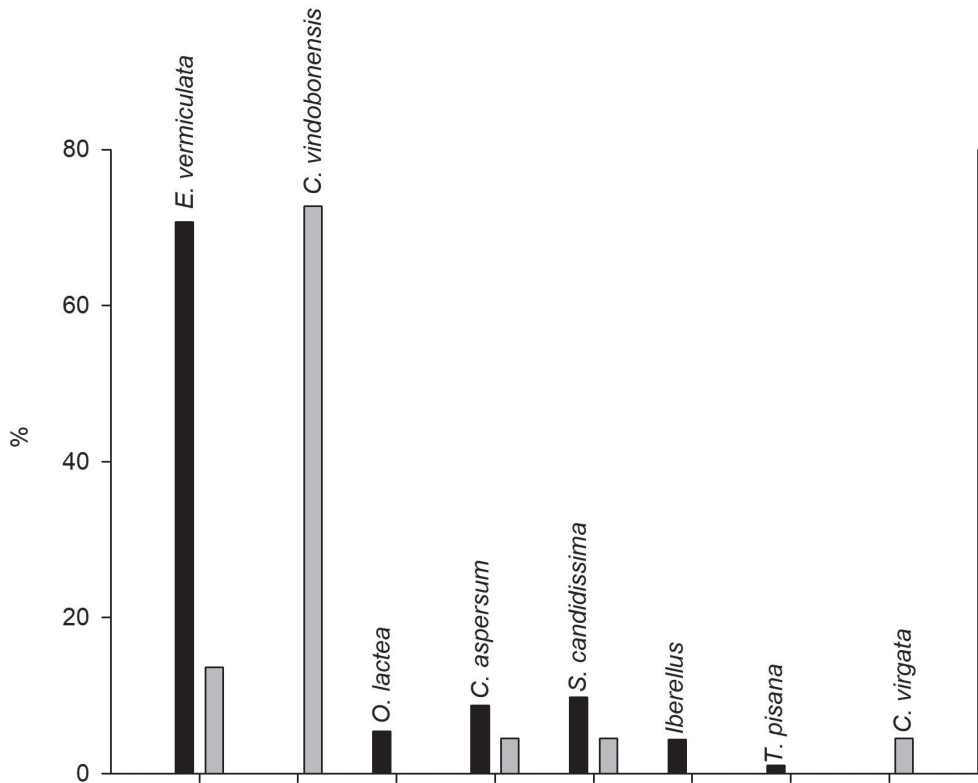


Figure 3. Proportions (in %) of shells used by *Rhodanthidium sticticum* (black columns) and *Rhodanthidium septemdentatum* (grey columns).

(Czech Republic and Slovakia) (11 of 17 nests examined) than in Spain (0 of 6 nests examined). Overall, we obtained 26 adult bees, 15 males and 11 females (M/F sex ratio: 1.4).

Shell choice. All nests from the Czech Republic and Slovakia were built in shells of *C. vindobonensis*, whereas nests from Spain were found in *Eobania vermiculata* (O. F. Müller) (3), *Sphincterochila candidissima* (Draparnaud) (1), *Cerneuella virgata* (Da Costa) (1), and *Cornu aspersum* (O. F. Müller) (1) shells (Fig. 3).

Shell manipulation. Females of *R. septemdentatum* do not move shells. All marked shells from our experiment in Prokopské údolí remained in place with no nesting on the ground surface, and only one shell placed near the centre of the nesting site (group A) was found under the stone with a nest of *R. septemdentatum*. However, we found five unmarked shells with nests under the stones on the same nesting site and suspected that the shell probably fell under the stone because of the climatic conditions before the nesting season of *R. septemdentatum*; alternatively, the space between the stones was utilized as a shelter by snails.

Life cycle. We dissected five nests in September 2017. All of them contained adult bees inside their cocoons. We also found adults in two nests collected during the winter of 2017/2018. In the spring of 2018, 16 young larvae from nine nests were transferred

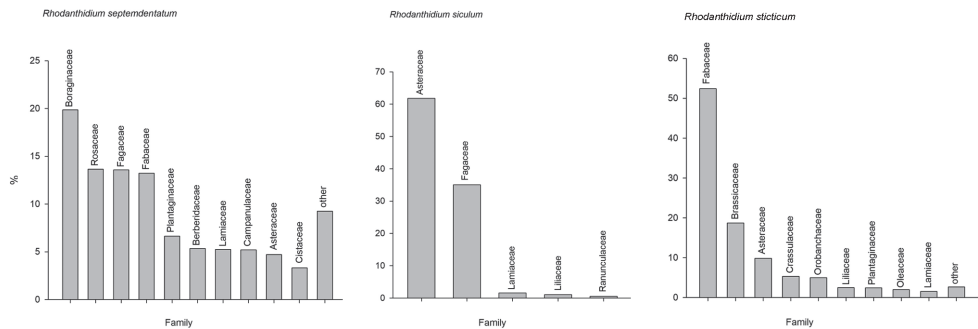


Figure 4. Proportions (in %) of pollen grains of plant families in studied nests of three species of *Rhodanthidium*.

with their pollen and nectar provisions to microtubes. The feeding larval stage lasted 5–8 weeks. Pupation occurred during July and August, and adults eclosed 2–4 weeks after pupation. Five larvae did not pupate and died during the winter. We conclude that *R. septemdentatum* overwinters in the adult stage in both study regions.

Nest associates. There were no nest associates with any of the *R. septemdentatum* nests.

Pollens collected. We analysed pollen samples from five nests from the Czech Republic. We recorded 41 pollen types from 22 plant families. Of these, 13 pollen types representing nine families were recorded in proportions higher than 10%. The most abundant pollen types were of the families Boraginaceae (20%, mostly *Echium vulgare*), Rosaceae (14%, mostly *Rubus* and *Filipendula*), Fagaceae (13%, mostly *Fagus sylvatica*), Fabaceae (11%, mostly *Cytisus*) and Plantaginaceae (7%) (Fig. 4 and Suppl. material 3: Table S3). Individual nests usually contained a mixture of pollen types from unrelated families. Only one nest contained a dominant pollen type (71% *Echium vulgare* pollen). The other nests contained 4–18 pollen types, of which only 2–5 represented more than 10% of the grains identified. These results indicate that *R. septemdentatum* is widely polylectic, and individual females do not specialize on any particular pollen source.

Rhodanthidium sticticum (Fabricius)

Material examined. 95 nests from various locations in Catalonia, north-eastern Spain (Suppl. material 2: Table S2).

Nest structure. The nests of this species have a vestibular cell and one (rarely two) brood cells. The closing plug was made of resin mixed with sand particles and sometimes fragments of snail shells (Fig. 2C). In most cases (62 nests), the closing plug was close to the aperture, but sometimes it was built a few mm inside the shell (33 shells). The vestibular cell was not delimited by a basal partition (Fig. 2D) and was loosely filled with mineral fragments, soil particles and plant debris (Fig. 2D, E). Most nests (90) had only one brood cell. Five nests contained two brood cells, and one

nest contained three brood cells. Overall, we obtained 76 adult bees, 44 males and 33 females (M/F sex ratio: 1.3).

Shell choice. Most nests (67) were built in shells of *E. vermiculata* (65). Other nests were built in shells of *S. candidissima* (9), *C. aspersum* (8, two of which juveniles), *Otala lactea* (O. F. Müller) (5), *Iberellus* sp. (4), and *Theba pisana* (O. F. Müller) (2) (Fig. 3). Multicell nests were found in *E. vermiculata* (two cells) and *O. lactea* (3 cells).

Shell manipulation. Most nests were found in shells hidden within stone walls or under stones. However, despite many hours of observation, we never observed any females dragging or hiding shells.

Life cycle. Eleven larvae from 21 nests collected in 2018 were transferred with their pollen nectar provisions into microtubes 4–10 days after collection. The feeding larval period lasted 3–6 weeks, and the pupal stage lasted 2–4 weeks. Adult eclosion occurred in July and August. Some larvae did not pupate and died during the autumn/winter.

Nest associates. We recorded parasitism by the ruby wasp *Chrysura refulgens* (Spinola) in two nests from Cap Ras (Girona) and by *Chaetodactylus* cf. *anthidii* mites in one nest from Sta. Margarida de Montbui (Barcelona). Overall, the parasitism rate in the nests examined was 3.03%. In addition, the three nests from Lleida (Lleida) and two nests from Òdena (Barcelona) contained low numbers of *C. cf. anthidii*, which did not cause the death of the bee.

Pollens collected. We analysed pollen samples in eight nests from Spain. We recorded 30 pollen types from 19 plant families. Of these, eight pollen types from six plant families were found in proportions greater than 10%. Most pollen grains identified (52%) were of the family Fabaceae (mostly *Cytisus* but also *Trifolium repens*), followed by Brassicaceae (19%) and Asteraceae (10%). Individual nests tended to be provisioned with a dominant (>50%) pollen type: *Cytisus* pollen was dominant in five nests, Brassicaceae pollen in two nests, and *Trifolium repens* pollen in one nest (Fig. 4 and Suppl. material 3: Table S3). These results indicate that *R. sticticum* is a polylectic species with a preference for collecting Fabaceae pollen and that females show a certain level of specialization, probably conditioned by the dominant pollen types in each locality.

Rhodanthidium siculum

Material examined. Two nests from Sicily.

Nest structure. The nests of this species contained only one brood cell. The vestibular space had no inner partition and, unlike the two previous species, was not filled with debris (Fig. 2G). The closing plug was made of resin with small fragments of snail shells and sand particles (Fig. 2F).

Shell choice. One nest was built in an *E. vermiculata* shell, and the other was built in a *T. pisana* shell.

Life cycle. In May, both nests contained young feeding larvae. Adult eclosion occurred in August.

Nest associates. No nest associates were recorded for this species.

Pollens collected. We analysed pollen from one nest. We identified nine pollen types from five plant families. The main plant family was Asteraceae (62%, mostly *Anthemis arvensis* but also *Centaurea jacea*), followed by Fagaceae (32%, mostly *Castanea*) (Fig. 4 and Suppl. material 3: Table S3). These results indicate that this species is also polylectic.

Rhodanthidium infuscatum

Material examined. Four nests from Spain. We found one nest in the city park in Castelldefels (Spain). The snail shell was found in a stone wall, and there were two cocoons, with hatched bees and partitioning in the nest. The structure of the nest was similar to that of the nest of *R. septemdentatum* but did not contain filling in the first empty cell. The other three records were collected in Spain by P. L. Scaramozzino. Two nests contained two individuals, and the third nest contained four individuals (mean 2.5 ± 0.5 SD).

Nest structure. The nests contained 2–4 brood cells and one vestibular cell. Both the brood cells and the vestibular cells were delimited by resin partitions (Fig. 2H). The vestibular cell was not filled with debris. The closing plug was made of resin mixed with small sand particles. Overall, we obtained 8 adult bees, 6 males and 2 females.

Shell choice. The nest found in Castelldefels was built in an *Iberellus* sp. shell and nests from Llanca (Girona) in *E. vermiculata* shells.

Nest associates. No nest associates were recorded for this species.

Discussion

The four species of *Rhodanthidium* studied build their nests in snail shells and use similar nesting materials, but the structures of their nests differ. All four use large snail shells, and the number of brood cells is inversely related to body size. The larger species, *R. septemdentatum*, *R. sticticum* and *R. siculum*, usually build one cell, sometimes two, per nest. By contrast, *R. infuscatum* (body length: 9–11 mm; Kasperek 2019) builds 2–4 brood cells per nest. Information on the number of cells per nest in this species was hitherto unknown (Pasteels 1977; Ortiz-Sánchez 1990). Most nests from Spain were built in shells of *E. vermiculata*, and most nests from Central Europe were found in similar-sized *C. vindobonensis*. Both these species are similar in size, shape and aperture diameter, and are numerous in steppic habitats. We suppose that nests of these species can also be found in the shells of other large genera, such as *Cepaea* Held and *Helix* Linnaeus. Although all four species are specialized in the use of snail shells as nesting substrates, *R. sticticum* has also been recorded nesting in linear cavities (paper tubes; Bosch et al. 1993).

All four species use fragments of shells, small stones and grains of sand pasted with resin as material for the closing plug, and all nests studied had a long vestibular space

Table 1. Comparison of main characters of nesting biology of four European *Rhodanthidium* species.

Character / Species	<i>R. infuscatum</i>	<i>R. septemdentatum</i>	<i>R. siculum</i>	<i>R. sticticum</i>
Brood cells per nest	2–4	1–2	1	1 (2)
Closing plug	resin + soil particles	resin	resin + shell particles + sand	resin + soil particles
Septa between brood cells	yes	yes	no	no
Filling	no	yes	no	yes
Individual pollen specialisation	N/A	no	no	yes
Moving shells	N/A	no	yes	no

between the plug and the outermost brood cell (Table 1). However, we observed some structural differences among species (Table 1). First, *R. septemdentatum* and *R. infuscatum* build partitions between the outermost brood cell and the closing plug, whereas *R. sticticum* and *R. siculum* do not. Second, *R. septemdentatum* and *R. sticticum* fill the vestibular space with debris, whereas the other two species do not. Therefore, our study provides new information on the behavioural differences across closely related species (*R. septemdentatum* and *R. infuscatum* nests are considered indistinguishable; Pasteels 1977). Interestingly, the nest structure of *R. sticticum* nests built in paper tubes (1–2 cells per nest, lacking a partition between the brood cells and the plug and vestibular space filled with debris; fig. 1 in Bosch et al. 1993) fully coincides with the structure that we observed in nests built in snail shells. The lack of cell partitions is an unusual trait among cavity-nesting megachilid bee species, the vast majority of which build nests with clearly delimited brood cells (e.g., Bosch et al. 1993; Vicens et al. 1993; Müller 2021). It is usually known in one or several species in a group, e.g. *Heriades spiniscutis* (Cameron) is the only species of the genus with known nests without partitions (Michener 1968); *Osmia brevicornis* (Fabricius) does the same (Radchenko 1979, in the study reported as *Metalinella atrocoerulea* Schilling). Although most species of crabronid wasps of the genus *Pemphredon* create nests with partitions in dead wood or plant stems (Blösch 2000), *Pemphredon fabricii* (Müller) nesting in reed stalks and galls creates nests without partitions and female provisions the smallest larvae (Bogusch et al. 2018). Another unusual trait among cavity-nesting megachilid bees is the filling of the vestibular space with loose debris. Bees nesting in empty snail shells usually do not use debris, but several species with well-described nesting behaviour are exceptions (*Osmia bicolor* Schrank and *O. rufobirta* Panzer) (Bellmann 1981; Müller et al. 2018; Heneberg et al. 2020).

Erbar and Leins (2017) reported that *R. siculum* created 1–2 brood cells per nest that were not separated by a partition. The nests studied by us contained the closing plug and pollen inside the shell behind the plug. As Erbar and Leins (2017) did not describe nest structure, this study is the first to describe the nest structures of this species. *R. septemdentatum* and *R. sticticum* nests are known to contain one or two brood cells separated by a transverse partitioning from resin, and the closing plug is made of grains of sand, small stones or plant residues glued together with resin. Grandi (1961) also described the nest construction of *R. septemdentatum* in the snail shell of *T. pisana*: the shell had the closing plug made from pieces of shells glued with resin, followed by a cell filled with various materials (small stones, sand grains, fragments of dry leaves,

bark and moss), a resin layer and then a brood cell with pollen. We confirm these observations provided by both authors. Nests of *R. sticticum* had the space behind the closing plug filled with small stones and plant pieces, followed by pollen with eggs or larvae without any partitions.

Consistent with previous studies (Pasteels 1977; Kasperek 2019), all nests that we studied in the field were placed under stones or inside stone walls. Despite many hours of observing *R. septemdentatum* and *R. sticticum* nesting females, we never observed any significant manipulation or transportation of shells. Instead, females appeared to choose shells that were already hidden under stones or in spaces in stone walls. This was confirmed by our manipulative experiment with shells of *C. vindobonensis* in Prokopské údolí. In contrast, Erbar and Leins (2017) provided a detailed description of *R. siculum* females transporting and burying nesting shells, usually beneath a plant. Importantly, the *R. siculum* population studied by Erbar and Leins (2017) nested in a sandy area with few stones. Future study of the nesting behaviour of *R. siculum* in stony areas and that of *R. septemdentatum* and *R. sticticum* in sandy areas could help determine whether shell manipulation is a plastic behavioural trait conditioned by the characteristics of the nesting environment.

Parasitism rates were low (3.4% of the cells obtained). We found *C. refulgens* in two nests of *R. sticticum*. *Chrysura refulgens* has been previously recorded from *R. septemdentatum* nests (Xambeau 1896; Friese 1911; Bogusch, unpublished observations in Greece) and probably parasitizes other *Rhodanthidium* species nesting in snail shells (Berland and Bernard 1938), as well as *O. bicolor*, another snail-nesting species (Strumia 1997). *Chrysura refulgens* occurs in southern Europe but does not reach Central Europe (Agnoli and Rosa 2019). We also found three *R. septemdentatum* nests with *Chaetodactylus* cf. *anthidii* (Klimov and O'Connor 2008). In one of these nests, the number of mites was high, and the bee did not develop. The other two nests contained few mites, and the bee larva had developed and spun its cocoon.

Rhodanthidium are polylectic bees (Bosch et al. 1993; Müller 1996; Erbar and Leins 2017; Westrich 2018; Kasperek 2019). Previous observations have indicated that *R. septemdentatum* females collect pollen for their brood primarily from the Fabaceae and Lamiaceae families (Kasperek 2019). Our results show that Boraginaceae, Rosaceae and Fagaceae pollen is also preferred. Bosch et al. (1993) found mostly *Cistus* and *Quercus* pollen in nests of *R. sticticum*. In our study, most of the pollen was from Fabaceae, followed by Brassicaceae and Asteraceae. *R. siculum* is known to collect pollen from Asteraceae and Fabaceae (Erbar and Leins 2017). In addition to Asteraceae, we also found Fagaceae pollen. To the best of our knowledge, the origin of the pollen collected by *R. infuscatum* remains unclear. We found that *R. sticticum* and *R. septemdentatum* are both polylectic, but the pollen preferences of individual females significantly differ. Each female of *R. septemdentatum* collected pollen from more species of unrelated plants (Boraginaceae, Rosaceae, Fagaceae, Fabaceae and Plantaginaceae in our surveys) and probably tracked the food supply, similar to *R. siculum* (Erbar and Leins 2017). Compared with this species, females of *R. sticticum* collected pollen from one dominant pollen source, which always made up more than half of all the pollen grains in the nest.

This pollen source differed among localities and among nesting females in one locality and usually belonged to the families Fabaceae, Brassicaceae and Asteraceae. Although we cannot comment on the generality of this individual specialization, our findings indicate that additional studies are needed to examine pollen preferences in both species.

Based on the phylogeny of *Rhodanthidium* (Litman et al. 2016; Kasperek 2019), all species of the subgenus *Rhodanthidium* likely nest in snail shells. According to several authors, a separate subgenus might be warranted for *R. infuscatum* based on its morphological differences (Michener 2007; Kasperek 2019). However, the nest structure of this species is similar to that of its relatives, except for the higher number of brood cells per nest, which appears to be related to the smaller body size of this species. *Rhodanthidium sticticum* and *R. siculum* are morphologically similar, but they differ in nest structure and possibly in nest manipulation (shell burying in *R. siculum* but not in *R. sticticum*) and possibly in pollen preferences (individual specialization in *R. sticticum* in contrast to unspecialized in *R. siculum*). Based on nest structure, *R. septemdentatum* combines the characters of the nesting biology of *R. siculum* and *R. sticticum*, but the main difference is in the presence of partitions or septa between the brood cells or between the empty cell at the closing plug and the first brood cell. Based on morphological traits (Kasperek 2019) and nest structure, *R. septemdentatum* appears to be closer to *R. infuscatum* than to *R. siculum* and *R. sticticum*. According to Litman et al. (2016), the genus *Rhodanthidium* belongs to the *Dianthidium* Cockerell clade, which includes genera that use resin to build their nests, whereas *Afranthidium*, the other genus nesting in snail shells, belongs to the *Anthidium* Fabricius clade, indicating that this behavioural trait evolved at least twice independently in tribe *Anthidiini*.

The majority of bees nesting in snail shells belong to the tribe *Osmiini*. In contrast to *Rhodanthidium*, most of these species use masticated plant leaves or mud to build their nest, but species of the genus *Protosmia* use resin (Müller et al. 2018). Many snail-nesting *Osmiini* have been reported to move their nest shells, and some are known to camouflage them with plant matter or cover them with pine needles or small twigs (e.g., *Osmia bicolor* and *O. rufohirta* Latreille; Bellman 1981; Vereecken and Le Goff 2012; Müller 2021). This behaviour has not been observed in *Rhodanthidium*, and the only species known to bury the shell nest is *R. siculum* (Erbar and Leins 2017). In contrast to *R. sticticum* and *R. siculum*, all snail-nesting bees of the tribe *Osmiini* build partitions between brood cells. Most species nesting in empty shells occur and nest in spring and overwinter as adults (Bellmann et al. 1981; Müller et al. 2018; our study). In Central Europe, only *Osmia spinulosa* (Kirby) nesting later in summer overwinters in prepupal stage (see Müller 1994).

Conclusions

We describe differences in the nesting biology of four closely related species belonging to the same subgenus *Rhodanthidium* (genus *Rhodanthidium*). In general, the nesting biology of all four species is quite similar. All species select shells of larger gastropod

species, collect pollen from multiple plant species, and use resin usually mixed with small soil or shell partitions for making closing plugs and partitions inside the nest. The main differences are in making a partition between the intercalary cell and first brood cell-nests of yellow-coloured species *R. infuscatum* and *R. septemdentatum* include partitions, while nests of orange-coloured species *R. siculum* and *R. sticticum* do not. Only *R. siculum* buries shells with nests in the ground (Erbar and Leins 2017), while *R. septemdentatum* and *R. sticticum* use hidden shells under stones or in stone walls for their nesting. All species are polylectic but individuals of *R. sticticum* show preferences. Using resin in nest supports the position of the genus *Rhodanthidium* in the *Dianthidium* clade as indicated Litman et al. (2016). Additional studies are needed, especially for the species *R. infuscatum*, which is the rarest of the four species studied (Kaspárek 2019). *R. sticticum* and *R. septemdentatum* are common species that form large local populations in southern Europe (Torné-Noguera et al. 2014; Romero et al. 2020) and the latter occurs in steppe habitats of conservation interest in central Europe (Bogusch et al. 2019, 2020).

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Appendix I. Description of sites surveyed

CZECH REPUBLIC

Prokopské and Radotínské údolí Nature Reserves in Prague. This area is occupied by hilly steppic grasslands on limestone subsoil, many snail species occur there and a larger amount of empty snail shells is available on the ground surface.

SLOVAKIA

Devínská Kobyla. The site is near the capital Bratislava, on a south-west slope of the hill. This area is occupied by hilly steppic grasslands on limestone subsoil, many snail species occur there and a larger amount of empty snail shells is available on the ground surface.

SPAIN

Lleida. The various sites in Lleida (Juneda, Castellans, Alamús, Aspa, Arbeca) were located in areas occupied by orchards and patches of Mediterranean scrubland vegetation (see Bogusch et al. 2020). Most nests were found in patches of natural habitat surrounding almond orchards. Nests were found within stone-walls and under stones on the ground.

Girona. The two sites in Girona (Cap Ras and Castell de Quermançó) are rocky areas covered by sparse Mediterranean scrubland. The *Rhodanthidium* nests were found within a collapsed stone wall, under the dry basal leaves of Agave plants and under a stone at the base of a bush.

Barcelona. The Garraf Natural Park comprises 123 km² of garrigue-type Mediterranean scrubland dominated by *Quercus coccifera*, *Rosmarinus officinalis* and *Thymus vulgaris* with sparse urban housing and long-time abandoned fields delimited by dry-stone walls.

The Òdena and Sta. Margarida de Montbui sites are located in rural areas of extensive agriculture with wheat fields, old almond orchards and olive groves. All nests were found in field margins and along dirt roads.

ITALY

Sicily. The two sites in Sicily where the *R. siculum* nests were found on a sandy habitat near the sea near Lido di Noto.

Supplementary material 1

Table S1. List of the localities, where nests of *Rhodanthidium* were studied

Authors: Lucie Hostinská, Jordi Bosch, Pier Luigi Scaramozzino, Petr Bogusch

Data type: table of localities (excel table)

Explanation note: This table contains all information to the localities of our studies.

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Link: <https://doi.org/10.3897/jhr.85.66544.suppl1>

Supplementary material 2

Table S2. List of all studied nests

Authors: Lucie Hostinská, Jordi Bosch, Pier Luigi Scaramozzino, Petr Bogusch

Data type: shells studied (excel table)

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Link: <https://doi.org/10.3897/jhr.85.66544.suppl2>

Supplementary material 3

Table S3. Pollen contents of nests

Authors: Petr Kuneš, Petr Bogusch

Data type: pollen contents (excel table)

Explanation note: Pollen contents of nests of *Rhodanthidium septemdentatum* (yellow), *R. siculum* (blue) and *R. sticticum* (green). Pollen types with 50% and more in one nest are marked in red, those with 10% and more in one nest are marked orange.

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