**Univerzita Hradec Králové**

**Přírodovědecká fakulta**

**Katedra biologie**

# Ekologie samotářských včel hnízdících v ulitách plžů a jejich vliv na úbytek stepních druhů

Disertační práce

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## Zadání dizertační práce

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Prohlašuji, že jsem dizertační práci vypracovala samostatně a že jsem v seznamu použité literatury uvedla všechny prameny, z kterých jsem vycházela.

V Hradci Králové dne 23. 5. 2022 Mgr. Lucie Hostinská

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Cílem této práce bylo studium druhů včel hnízdících v prázdných ulitách terrestrických plžů a vyhodnocení jejich zastoupení na přirozených stepních lokalitách a přilehlých postindustriálních stanovištích. Dále byla provedena studie o změnách početnosti ulitových druhů včel v porovnání se stepními včelami hnízdícími v zemi, s cílem zjistit, jaký management je vhodný pro zachování stepí a stepních druhů ve středoevropské krajině. Zatímco se včely hnízdící v ulitách rozšířily na více lokalit a zvětšily své populace, včely hnízdící v zemi z fauny ČR ubývají. Důvodem změn je nepříznivý management stepí způsobující jejich postupné zarůstání. To vede k několika aspektům – větší šíření plžů preferujících zarostlé lokality a s tím související vyšší dostupnost hnízdního zdroje pro ulitové druhy a zároveň absence holých půdních ploch, a tedy úbytek hnízdních zdrojů pro druhy hnízdící v zemi. Dalším důvodem rozšíření ulitových druhů včel je postupné stěhování plžů i na různá antropogenní stanoviště a schopnost některých ulitových druhů včel je na tato místa následovat. Dále byla zkoumána specializace jednotlivých druhů včel na ulity konkrétních druhů plžů, vzhled jejich hnízd, pylové preference a parazitoidi a kleptoparaziti u jednotlivých druhů. Zaměřili jsme se především na druhy vyskytující se v ČR a čtyři evropské druhy rodu *Rhodanthidium*, krom toho jsme popsali hnízdní biologii jihoevropských druhů *H. fertoni* a *O. ferruginea*. V rámci výzkumů vznikl i první ucelený přehled hmyzu, který přezimuje v prázdných ulitách ve střední Evropě.

Klíčová slova:

Megachilidae, *Osmia*, *Rhodanthidium*, hnízdní struktura, ulita, step, kleptoparazit, parazitoid

Annotation:

HOSTINSKÁ, L. *Ecology of solitary bees nesting in empty snail shells and their influence on decrease of steppe specialists*. Hradec Králové 2022. Dissertation at Faculty of Science University of Hradec Králové. Dissertation Supervisor Petr Bogusch, 50 p.

The aim of this survey was to study the bees nesting in empty shells of terrestrial gastropods and to evaluate their abundances in natural steppic habitats and neighbouring postindustrial sites. Part of this survey was also the study on the changes of abundances of these species in comparison with bees nesting in soil, with the main aim to find out the appropriate management for conserving the steppes and steppic species in central European landscape. While the bees nesting in empty gastropod shells colonized new areas and formed stronger populations, the ground-nesting bees are disappearing from the fauna of the Czech Republic. The reasons of these changes are unfavourable nature conservation management practices resulting in the successional shifts of steppic formations towards shrubs and mesic grasslands. It leads to several aspects – greater distribution of snails preferring overgrown habitats and the related increase of the number of available gastropod shells (i.e. nesting resources for shell-nesting bees) and the absence of bare ground (i.e. loss of nesting resources for ground-nesting bees). Another reason for spreading of shell-nesting bees is the gradual settling of several snail species in various anthropogenic sites and the ability of some shell-nesting bees to follow them to this places. Also the preferences of bees nesting in empty gastropod shells was studied, as well as the morphology of nests, pollen specializations, and biology and host specificity of the parasitoids and cleptoparasites. We focused especially on the species distributed in the Czech Republic and four European species of the genus *Rhodanthidium*. We also described the nesting biology of south-european species *Hoplitis fertoni* and *Osmia ferruginea*. The complete profile of insects that use empty terrestrial snail shells during the winter period in Central Europe was created during our research, too.

Key words:

Megachilidae, *Osmia*, *Rhodanthidium*, nest structure, snail shell, steppe habitat, cleptoparasite, parasitoid

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# 1 ÚVOD

Disertační práce je zaměřena na samotářské včely hnízdící v prázdných ulitách plžů. Teoretická část obsahuje čtyři hlavní kapitoly. Ta první, s názvem Hnízdní specializace včel, stručně shrnuje způsoby hnízdění včel v různých substrátech a zmiňuje také hnízdní parazity, kteří ke kladení vajíček využívají hnízda jiných druhů včel.

Kapitola Včely v ulitách poukazuje na systematické zařazení včel využívajících k hnízdění ulity a popisuje různé způsoby stavby hnízda a manipulace s ulitou u včel náležících do různých druhů a rodů s důrazem na včely vyskytující se v Palearktu. Dále jsou zde vyjmenovány druhy vyskytující se v České republice, se kterými podrobně seznamují následné podkapitoly. V textech u jednotlivých druhů jsou shrnuty doposud známé informace z odborné literatury i poznatky z našich výzkumů.

Kapitola Paraziti v ulitách pojednává zejména o žahadlových blanokřídlých kleptoparazitech a parazitoidech, kteří se vyskytují u ulitových včel v České republice. Konkrétně se jedná o kukaččí včelu *Stelis odontopyga* Noskiewicz, 1926, tři druhy zlatěnek rodu *Chrysura* Dahlbom, 1845 a drvenku *Sapyga quinquepunctata* (Fabricius, 1781). Okrajově zmiňuje i další zástupce řádů Hymenoptera, Diptera a Coleoptera parazitující v ulitových hnízdech samotářských včel. Informace z literatury jsou zde opět doplněny o naše vlastní poznatky.

Kapitola Další blanokřídlí hnízdící v ulitách poukazuje na jiný blanokřídlý hmyz kromě včel, který může k hnízdění využívat ulity, a to buď jen fakultativně, nebo přímo obligátně. Konkrétně se věnuje šesti druhům hrabalek čeledi Pompilidae a třem druhům hrnčířek rodu *Leptochilus* de Saussure, 1853 (čeleď Vespidae). Okrajově zmiňuje také některé zástupce mravenců rodů *Myrmica* Latreille, 1804 a *Temnothorax* Mayr, 1861 (čeleď Formicidae). Převážná většina zde uvedených druhů se vyskytuje i na území České republiky.

V Přílohách je představeno šest článků publikovaných v časopisech s impakt faktorem. Během studia jsme se zaměřili na několik hlavních témat. Zjišťovali jsme specializaci včel na ulity konkrétních druhů plžů, hostitelskou specificitu zde parazitujících zlatěnek a dalších parazitoidů a kleptoparazitů, zkoumali jsme ekologické preference ulitových druhů včel a také jsme sledovali změny výskytu druhů hnízdících v ulitách v porovnání s druhy, které hnízdí v zemi. Mimo to nás zajímalo, jak přesně vypadají hnízda v ulitách jednotlivých druhů včel, v jakém stadiu tyto včely přezimují nebo které rostliny využívají jako zdroj pylu a nektaru.

Výzkumy jsme orientovali především na druhy vyskytující se v ČR a dále na evropské druhy rodu *Rhodanthidium* Isensee, 1927, okrajově pak i na další ulitové druhy.

Nejprve jsme se zaměřili na vybrané skupiny hmyzu a pavouky na přírodních stanovištích (stepi a lesostepi) a blízkých postindustriálních biotopech. Cílem bylo zjistit, zda k rozšíření hmyzu využívajícího ulity z přírodních stanovišť na antropogenní s obdobnými podmínkami stačí pouze samotný výskyt těchto prázdných ulit. Výzkum spočíval v zimním sběru ulit, jejich roztřídění dle druhů plžů a ponechání v laboratorních podmínkách. Vylíhlý hmyz jsme pravidelně odebírali, fixovali v ethanolu a následně určili. Výsledky výzkumu vůbec poprvé přinesly rozsáhlejší přehled středoevropského hmyzu využívajícího prázdné ulity k přezimování a hnízdění (Bogusch a kol. 2019).

Náš další výzkum se už zaměřil přímo na včely a byl postaven na manipulativním experimentu, kdy jsme na zvolené přírodní i antropogenní lokality s přirozeně se vyskytujícími ulitami umístili ulity barevně označené. Na místě jsme je ponechali po dobu hnízdění studovaných druhů. Po následném sesbírání byly ulity rozebrány a v případě nalezení hnízda byl zaznamenán druh plže, popsána struktura tohoto hnízda, dospělí jedinci byli fixováni v ethanolu, juvenilní přemístěni do mikrozkumavek k dolíhnutí, dále jsme z hnízda odebrali vzorky pylu a případných plísní a odeslali je na analýzu. Zmíněný experiment navíc přinesl odpověď na otázku, zda mohou být prázdné ulity na lokalitě limitujícím zdrojem pro specializované druhy žahadlových blanokřídlých (Heneberg a kol. 2020).

Jelikož druhy včel hnízdící v ulitách se v posledních letech nalézají mnohem častěji než dříve a v novém červeném seznamu (Straka & Bogusch 2017) většina z nich již v žádné z kategorií ohroženosti není, zaměřili jsme se na zmapování výskytu těchto druhů a jejich parazitů v minulosti (do roku 1990) a v současnosti. Na základě faunistických dat jsme zpracovali mapy rozšíření druhů, vyhodnotili jejich možné areály rozšíření na území České republiky v minulosti a v současnosti a toto srovnali se včelami rodu *Eucera* Scopoli, 1770, které se vyskytují také především na stepních stanovištích, ale hnízdí v zemi (Bogusch a kol. 2020a).

Samotářské včely slouží jako důležití opylovači volně rostoucích i člověkem pěstovaných rostlin. Některé druhy těchto samotářských včel tvořící silné populace na otevřených stanovištích v Evropě hnízdí v prázdných ulitách plžů. Důležitost přírodě blízkých stanovišť s přítomností prázdných ulit pro přítomnost v ulitách hnízdících včel (a vos) jsme zkoumali na přírodě blízkých i ruderálních stanovištích sousedících s mandloňovými sady nedaleko města Lleida v severovýchodním Španělsku. Naším cílem bylo také zjistit, na jakých typech stanovišť se ulitové včely vyskytují a jaké jsou rozdíly mezi jejich zastoupením podle

typu lokality a druhu ulity. Dále nás zajímala také hnízdní bionomie jednotlivých druhů. Na lokalitách jsme provedli manipulativní experiment s nastraženými barevně označenými ulitami, získaná data jsme pak pro každou lokalitu porovnali s nálezy hnízd včel v přirozeně se vyskytujících ulitách (Bogusch a kol. 2020b).

Mezi samotářské včely hnízdící v ulitách ve Středomoří patří druhy *Hoplitis fertoni* (Pérez, 1891) a *Osmia ferruginea* Latreille, 1811. Na výše uvedených lokalitách u mandloňových sadů jsme získali větší množství hnízd od těchto dvou druhů. Jejich hnízdní biologie byla dosud popsaná jen částečně. Pro každý druh jsme zaznamenali hnízdní strukturu (např. počet hnízdních komůrek, materiál používaný na zátku a přepážky), preferovaný druh ulit a na základě pylových analýz také pylovou specializaci. V některých hnízdech jsme objevili parazity, a to povětšinou druhy, které u těchto včel nebyly doposud pozorovány (Bogusch a kol. 2020c).

V neposlední řadě jsme se zaměřili na evropské druhy rodu *Rhodanthidium*. O hnízdní biologii těchto druhů nebylo doposud příliš známo a většina publikovaných dat pocházela z hodně starých zdrojů. V rámci studií ulitových včel ve střední Evropě a ve Španělsku jsme se zaměřili na strukturu hnízd, preferované druhy ulit, parazity a pylovou specializaci všech čtyř druhů tohoto rodu, které hnízdí v prázdných ulitách (Hostinská a kol. 2021).

# 2 TEORETICKÁ ČÁST

## 2.1 Hnízdní specializace včel

Včely (Apiformes) hnízdí v rozmanité škále substrátů. Většina si staví hnízda v zemi, ale mnoho z nich si vytváří dutiny pro hnízdění ve dřevě či v měkké dřeni stonků, jiné druhy mají svá hnízda volně postavená a připevněná k podkladu, další využívají již existujících dutin, jako jsou dutá stébla, přirozené dutiny ve skalách či mezi kameny, staré broučí chodby, opuštěná hnízda jiných blanokřídlých, prázdné ulity plžů nebo staré hálky. Některé druhy kladou vajíčka do hnízd jiných druhů včel a svá vlastní si nestaví (Westrich 2018, Danforth a kol. 2019).

Většina hnízdících včel nanáší na stěny svých plodových buněk sekrety vlastních žláz, některé používají rostlinné oleje, mnoho včel z čeledi Megachilidae používá ke stavbě hnízda různé kombinace jílu, písku, kamínků a rostlinného materiálu, jako jsou listové úkrojky či rozžvýkaná listová pasta, okvětní plátky, rostlinné trichomy nebo pryskyřice (Michener 2007, Danforth a kol. 2019).

U hnízd vytvořených v zemi se konkrétní vzhled liší v závislosti na druhu včely. Obvykle je tvořeno hlavní, vertikální, chodbou, na kterou se napojují chodby vedlejší. Na konci každé z těchto vedlejších chodeb často bývá jediná hnízdní komůrka, někdy ale mohou být komůrky seskupeny dohromady. Vedlejší chodby mohou být v závislosti na druhu různě dlouhé a vedené z hlavní chodby pod různým úhlem. U některých druhů se vyskytuje jen jediná komůrka, hnízda dalších druhů obsahují i slepé chodby bez komůrek, samice některých druhů zase vynáší vyhrabanou zeminu na povrch, kde vchod do hnízda označují tzv. tumulem, případně komínem (Antoine & Forrest 2020). Některé druhy včel využívají také již v zemi existující dutiny (Macek a kol. 2010).

Hnízda ve dřevě jsou oproti podzemním hnízdům obvykle tvořena jedinou chodbou, ve které jsou v řadě za sebou vystavěny plodové buňky oddělené přepážkami. Vzhledově se mohou lišit podle druhu, většina je však závislá na tvaru již existující původní dutiny. Jen několik málo druhů si umí dutinu vykousat samo (Bogusch & Horák 2018).

Hnízda ve stoncích tvoří taktéž jediná chodba s plodovými buňkami uspořádanými lineárně. Některé druhy včel hnízdí v dutých stoncích (například rákosových stéblech), jiné si samy vykusují dutiny do měkké dřeně lodyh či větviček (Michener 2007, Mikát a kol. 2021).

Hnízda ve skalách u druhů, které si zde dutiny hloubí samy, mohou být tvořena hlavní chodbou větvící se na další chodby vedlejší, ve kterých jsou jednotlivé plodové komůrky umístěny lineárně. Podmínky k hnízdění jsou zde různé v závislosti na podkladu (písek, štěrk, jíl, spraš) a expozici. Krom skal mohou včely osidlovat také hliněné zdi a spáry v suchých kamenných zídkách. Některé druhy včel si chodby ve skalách hloubit neumí a využívají dutiny již vytvořené (Westrich 2018).

Druhy hnízdící v hálkách nejčastěji využívají staré hálky vytvořené zelenuškou *Lipara lucens* Meigen, 1830 (Chloropidae, Diptera) na stéblech rákosu obecného (*Phragmites australis*). Některé druhy hnízdí v hálkách obligátně, jiné pro stavbu hnízda využívají i jiné dutiny (Bogusch a kol. 2015, Astapenková a kol. 2017). Hnízdní komůrky jsou zde uspořádány lineárně (Bogusch a kol. 2015).

Ulitové včely vykazují velké rozdíly v hnízdění, ať už se jedná o preferovaný typ ulity, počet plodových buněk či prázdných komůrek nebo používaný materiál (např. Müller 2021). Některé druhy po vytvoření hnízda s ulitou již nemanipulují ani ji neskrývají, jiné ji naopak zakopávají nebo alespoň pokrývají rostlinným materiálem (Danforth a kol. 2019).

Hnízda některých druhů z čeledi Megachilidae mohou být umístěná na povrchu skal, zdí, stonků či listů a sestávají z několika buněk vytvořených například z pryskyřice, pryskyřice a kamínků, listové hmoty nebo jílu (Michener 2007). Například hliněná hnízda mohou být tvořena ze směsi písku a částeček zeminy a impregnované slinami (Michener 2007), pryskyřičná hnízda bývají vystavěna z pryskyřice a malých kamínků (Michener 2007, Bogusch & Horák 2018), někdy také s vtroušenými kousky kůry (Macek a kol. 2010).

Do skupiny Apiformes patří i kleptoparazitické druhy (tzv. kukaččí včely), které si nestaví hnízda, ale kladou vajíčka do hnízd jiných druhů samotářských včel (Wiesbauer a kol. 2020). Larva parazita se v tomto případě živí na potravních zásobách, které byly původně nashromážděny pro hostitelskou larvu, tedy pylem a nektarem (Michener 2007). V parazitované plodové buňce se vyvíjí pouze larva kukaččí včely, potomstvo hostitele je zneškodněno – buď je vajíčko ihned odstraněno již samicí kleptoparazita, nebo je vajíčko či vylíhlá larvička hostitele eliminována až samotnou kleptoparazitickou larvou (Antoine & Forrest 2020).

U sociálních druhů včel se vyskytují sociální paraziti (Michener 2007). Ti si uzurpují hnízda svých hostitelů, hostitelské dělnice pak vychovávají larvy parazita a někdy také zabíjejí svou původní (hostitelskou) královnu (Antoine & Forrest 2020).

## 2.2 Včely v ulitách

Na celém světě je odhadem 20 000 druhů včel zařazených do 7 čeledí (Michener 2007), v prázdných ulitách hnízdí včely pouze z čeledi Megachilidae. Tato čeleď zahrnuje celosvětově přes 4 000 druhů z více jak 70 rodů, které jsou řazeny do 7 tribů (Michener 2007, Ascher & Pickering 2020). Hnízdění v ulitách bylo v rámci čeledi Megachilidae pozorováno pouze u dvou tribů (Osmiini a Anthidiini) a celkem 7 rodů – 5 rodů z tribu Osmiini (*Ashmeadiella* Cockerell, 1897, *Hoplitis* Klug, 1807, *Osmia* Panzer, 1806, *Protosmia* Ducke, 1900, *Wainia* Tkalcu, 1980) a 2 rody z tribu Anthidiini (*Afranthidium* Michener, 1948, *Rhodanthidium*) (Müller a kol. 2018, Danforth a kol. 2019).

Největší množství druhů včel hnízdících v ulitách je u tribu Osmiini – celosvětově celkem 52 druhů patřících do 5 rodů a 14 podrodů. Z toho palearktických druhů tribu Osmiini je 43 (náležících do 4 rodů). Dá se tedy říci, že převážná většina včel z tribu Osmiini hnízdících v ulitách se vyskytuje v Palearktu. Ve většině případů se jedná o specialisty na hnízdění v ulitách, jen v několika málo případech mohou druhy využívat i jiné hnízdní zdroje (Müller a kol. 2018).

U tribu Anthidiini bylo pozorováno hnízdění v ulitách pouze u 4 palearktických druhů rodu *Rhodanthidium* (Erbar & Leins 2017, Baldock a kol. 2018, Westrich 2018, Romero a kol. 2020, Hostinská a kol. 2021) a 2 afrotropických druhů rodu *Afranthidium* (Gess & Gess 2008, 2014).

Mimo tyto dva triby existuje jediný záznam o hnízdění v ulitě u včely druhu *Megachile lefebvrei* (Lepeletier, 1841), která patří do tribu Megachilini a obvykle si staví hnízda v mezerách v kamenech a skalách, fakultativně však může využít také prázdné ulity (Müller a kol. 2018).

Hnízdění v ulitách je podrobně zdokumentováno zejména u palearktických druhů z tribu Osmiini, konkrétně se jedná o 4 rody (*Hoplitis, Osmia, Protosmia* a *Wainia*) a 43 druhů, z nichž 32 hnízdí v ulitách obligátně (nebo se to alespoň předpokládá) a 9 fakultativně, u 2 druhů zatím k podobnému závěru není dostatek informací (Müller a kol. 2018, Müller 2021). Z palearktických druhů z tribu Anthidiini bylo hnízdění v ulitách pozorováno u 4 druhů rodu *Rhodanthidium*, ve většině případů se dle dostupných informací jedná o hnízdění obligátní (Kasparek 2019, Hostinská a kol. 2021). Pouze u druhu *R. sticticum* (Fabricius, 1787) bylo pozorováno výjimečné zahnízdění i v jiném typu dutiny, konkrétně v papírových trubičkách.

Struktura těchto hnízd se shodovala se strukturou hnízd stavěných tímto druhem v ulitách (Bosch a kol. 1993).

Hnízdo v ulitě sestává z plodových komůrek oddělených od sebe přepážkami, vchod bývá často uzavřen silnější zátkou. Počet komůrek může být různý, často je jen jedna, u druhu *Osmia aurulenta* (Panzer, 1799) jich však bylo zaznamenáno až 17. Plodové buňky jsou většinou uspořádány za sebou, u větších ulit mohou být některé umístěny i vedle sebe (Müller 2021).

Přepážky mezi komůrkami i zátka mohou být z různých přírodních materiálů. Někdy jsou přírodním materiálem obložené i stěny plodových buněk (obzvlášť u větších ulit). Používaná hmota se mezi jednotlivými rody liší, dle dostupných informací používají některé zednice rodu *Hoplitis* jíl, který může být někdy smíchaný s malými kamínky (Bogusch a kol. 2020c, Müller 2021). Jiné druhy ze stejného rodu staví hnízda s použitím směsi listové pasty a zrn písku, případně používají okvětní plátky (Müller a kol. 2018). Zednice rodu *Osmia* používají ke stavbě přepážek nejčastěji pastu z rozžvýkaných listů (někdy také smíchanou s pískem). Uzávěr hnízda může sestávat z několika vrstev, kdy na poslední přepážku z rozžvýkaných listů navazuje zátka, která krom listové dužiny může obsahovat například i malé kamínky, úlomky ulit, částečky zeminy či písková zrna. Někdy je prostor mezi dvěma posledními přepážkami (neboli přepážkou a zátkou) z rozžvýkané listové hmoty vyplněn vrstvou hustě nahloučených kamínků a dalších drobných materiálů. Některé zednice rodu *Osmia*, které hnízdí v ulitách fakultativně (např. *O. bicornis* (Linnaeus, 1758), *O. cornuta* (Latreille, 1805) a *O. tricornis* Latreille, 1811), používají místo listové hmoty jíl. Druhy rodu *Protosmia* staví hnízdo pomocí pryskyřice, druhy rodu *Wainia* pomocí velkých kusů okvětních lístků, nebo zrnek písku slepených dohromady hmotou připomínající pryskyřici (Müller a kol. 2018, Müller 2021). Druhy rodu *Rhodanthidium* používají pryskyřici smíchanou s kousky písku, úlomky ulit a suchým rostlinným materiálem (Hostinská a kol. 2021).

Některé druhy s ulitou po zahnízdění manipulují, otáčí ji ústím dolů, přesouvají či zahrabávají, jiné ji nechávají na místě a nehýbou s ní. Samice některých druhů na ulitu po vytvoření hnízda nanesou pastu z rozžvýkaných listů, případně ji celou zakryjí (Müller 2021).

V České republice hnízdí obligátně v ulitách 6 druhů včel ze 2 rodů a 6 podrodů. Jsou to *Osmia* (*Allosmia*) *rufohirta* Latreille, 1811, *Osmia* (*Erythrosmia*) *andrenoides* Spinola, 1808, *Osmia* (*Helicosmia*) *aurulenta* (Panzer, 1799), *Osmia* (*Hoplosmia*) *spinulosa* (Kirby, 1802), *Osmia* (*Neosmia*) *bicolor* (Schrank, 1781) a *Rhodanthidium* (*Rhodanthidium*) *septemdentatum* (Latreille, 1809) (Macek a kol. 2010, Bogusch & Horák 2018, Bogusch a kol. 2020a, Müller 2021). V červeném seznamu z roku 2005 byly 4 z těchto 6 druhů uvedeny jako druhy ohrožené

(EN) – konkrétně se jednalo o druhy *O. rufohirta*, *O. andrenoides*, *O. bicolor* a *R. septemdentatum* (Straka 2005). V nejnovějším červeném seznamu z roku 2017 figuruje již jen jeden druh, *O. andrenoides*, a to jako druh kriticky ohrožený (CR) (Straka & Bogusch 2017).

Velikosti ulit našich plžů se pohybují v rozmezí od 1,5 mm do 4 cm (Horsák a kol. 2013), k hnízdění jsou však vhodné pouze schránky velkých plžů. Pro lepší přehlednost jsou dále v textu ve vztahu k hnízdění rozlišované ulity malé, střední a velké, toto označení však odkazuje pouze na rozsah velikostí ulit vhodných k hnízdění, nikoliv na rozsah velikostí všech ulit (nejen) u nás žijících plžů.

### 2.2.1 *Osmia* (*Allosmia*) *rufohirta* Latreille, 1811

Druh preferuje otevřená, nezarostlá místa (Bogusch a kol. 2020a) na vápenatém podloží. Osidluje vnitrozemské duny, skalnaté svahy, rozsáhle spásaná nebo ladem ležící vřesoviště, opuštěné lomy, ale také kamenité náspy silnic (Westrich 2018).

Hnízdí výhradně v prázdných ulitách plžů, z ulit vhodných k hnízdění si vybírá ty s malou až střední velikostí (Müller 2021), k hnízdění tedy preferuje schránky suchomilek *Xerolenta obvia* (Menke, 1828) nebo *Helicella itala* (Linnaeus, 1758) či páskovek např. rodu *Cepaea* Held, 1838 (Bellmann 1981, Bogusch a kol. 2019, Heneberg a kol. 2020), byl nalezen také v ulitách *Monacha cartusiana* (O. F. Müller, 1774) (Bogusch a kol. 2020a). Müller (2021) navíc uvádí i ulity plžů rodů *Bulimus* J. G. Bruguière, 1792, *Candidula* Kobelt, 1871, *Helicopsis* Fitzinger, 1833, *Pomatias* Studer, 1789, *Theba* Risso, 1826 nebo *Zebrina* Held, 1838.

Po spáření samice vyhledá prázdnou ulitu, kterou nejprve přemístí na vhodnější místo. Při transportu si pomáhá kusadly, kterými se přichycuje stébel trávy a dalších předmětů v okolí. Ulitu uchopí do nohou, pomocí kusadel se vzepře a přetočí. Ulitu takto přepraví na vzdálenost od 0,2 m do 1 m. Tam ji poté pokryje rozžvýkaným rostlinným materiálem a začne hnízdo zásobit pylem a nektarem (Bellmann 1981, Westrich 2018). Müller (2021) tvrdí, že k přemístění ulity před hnízděním dochází vzácně, častější je transport po uzavření hnízda, a to až do vzdálenosti 2,2 m. Westrich (2018) uvádí, že samice může přesouvat ulitu před započetím hnízdění i po dokončení hnízda.

*O. rufohirta* nevytváří před první plodovou buňkou (směrem dovnitř ulity) žádnou přepážku (Bellmann 1981). Ještě, než na zásoby naklade vajíčko, připraví si samice další rozžvýkanou rostlinnou pastu, kterou si nahromadí v místě budoucí přepážky na konci komůrky (Bellmann 1981, Westrich 2018). Prostor mezi pylovými zásobami a budoucí přepážkou má

délku 5–8 mm (Bellmann 1981). Následně na potravní zásoby umístí vajíčko a plodovou buňku uzavře (Bellmann 1981, Westrich 2018). Hnízda obvykle obsahují pouze jedinou hnízdní komůrku (Bellmann 1981, Müller a kol. 2018, Westrich 2018, Müller 2021).

Za přepážku z listové dužiny jsou nahromaděny drobné kamínky a hrudky zeminy, za kterými následuje zátka ze stejného materiálu jako předešlá přepážka (Bellmann 1981, Müller a kol. 2018, Westrich 2018). Uzávěr hnízda má tedy tři vrstvy, přičemž ty krajní, utvořené z rozžvýkané listové hmoty, uzavírají prostřední vrstvu z nashromážděného minerálního materiálu (Westrich 2018, Müller 2021). Prostor s kamínky mezi přepážkou a zátkou je široký 2–5 mm (Bellmann 1981). Krom listů může na rostlinnou pastu pro přepážku a zátku používat také okvětní plátky (Müller 2021).

Dokončené hnízdo je ukryto na bezpečné místo, např. pod vegetaci či kameny (Bellmann 1981, Müller a kol. 2018, Westrich 2018). Ulity mohou být také alespoň částečně zahrabány do země (Müller a kol. 2018, Bogusch a kol. 2020b, Müller 2021).

Dospělci se líhnou už koncem léta, zůstávají ale uvnitř hnízda a v ulitě přezimují (Bellmann 1981, Müller & Richter 2019).

Jedná se o polylektický druh preferující čeleď Fabaceae (Gogala 1999, Westrich 2018, Müller 2021), ale sbírající pyl a nektar také na rostlinách čeledí Asteraceae, Brassicaceae, Cistaceae, Campanulaceae, Boraginaceae, Convolvulaceae a Lamiaceae (Müller 2021).

Dle dosavadních pozorování se zde nevyskytuje žádný hnízdní parazit (Westrich 2018). Dle Bellmanna (1981) do hnízd klade vajíčka parazitoid *Chrysura cuprea* (Rossi, 1790), Wiesbauer a kol. (2020) uvádějí ještě druh *Chrysura dichroa* (Dahlbom, 1854).

### 2.2.2 *Osmia (Erythrosmia) andrenoides* Spinola, 1808

Jedná se o poměrně vzácný druh vyskytující se v teplejších oblastech (Müller & Richter 2019). Upřednostňuje extrémně kamenitá stanoviště, která jsou kvůli svému jižnímu působení obzvláště suchá a během léta velmi horká (Westrich 2018). Hnízdí na kamenitých suchých svazích, skalních stepích, zvětrávajících haldách kamení i štěrkových polích (Westrich 2018, Müller & Richter 2019).

Hnízda si staví výhradně v prázdných ulitách plžů (Gogala 1999, Müller a kol. 2018), vybírá si schránky malých až středně velkých druhů, např. *Cepaea hortensis* (O. F. Müller, 1774), *Cernuella neglecta* (Draparnaud, 1805), *Helix pomatia* Linnaeus, 1758, *Pomatias elegans* (O. F. Müller, 1774), *Theba pisana* (O. F. Müller, 1774), *X. obvia* nebo *Zebrina detrita* (O. F. Müller, 1774). Zahnízděné ulity jsou obvykle ukryty na alespoň částečně zastíněných

místech pod kameny či výčnělky skal, což by mohlo vysvětlovat výše zmíněnou výraznou preferenci skalnatých nebo kamenitých biotopů (Müller & Richter 2019, Müller 2021).

Ke stavbě hnízda je používána pasta z rozžvýkaných listů (Gogala 1999, Müller a kol. 2018, Westrich 2018, Müller & Richter 2019, Müller 2021). Hnízdo obsahuje od jedné do tří plodových buněk. Směrem do vnitřní části ulity není první komůrka oddělena žádnou stěnou (Müller & Richter 2019, Müller 2021). Směrem k ústí ulity jsou od sebe jednotlivé komůrky oddělené jednovrstevnými přepážkami vytvořenými z listové dřeně. Za poslední dokončenou plodovou buňkou následuje vrstva nahromaděných kamínků, úlomků zeminy a jiného drobného materiálu. Hnízdo je nakonec uzavřeno dvěma těsně po sobě následujícími přepážkami z rozžvýkaných listů. Povrch ulity není potřísněn skvrnami listové dužiny (Müller a kol. 2018, Müller & Richter 2019, Müller 2021). Ústí zahnízděné ulity často směřuje nahoru nebo do strany, což naznačuje, že samice s ulitou po ukončení hnízda nepohybuje (Müller & Richter 2019, Müller 2021).

Podrobná hnízdní biologie druhu byla dlouho neznámá (Westrich 2018), detailní informace přinesli Müller & Richter (2019), kteří prozkoumali 19 hnízd v ulitách *Z. detrita*. Jednotlivé plodové buňky jsou 13–17 mm dlouhé. Přepážky z rostlinné malty jsou po okrajích široké 0,4–0,5 mm, ve střední části 0,1–0,2 mm. Přepážka poslední komůrky bývá silnější, po okrajích s tloušťkou 0,5–1 mm (uprostřed 0,2–0,3 mm). Prostor s kamínky a dalším drobným materiálem za poslední vystavěnou komůrkou je dlouhý 4–13 mm. Následují dvě přepážky z rostlinné malty přímo za sebou, ta vnější bývá přímo v ústí ulity. Tyto přepážky jsou často v okrajových částech navzájem spojeny, zahrnují prostor o délce 0,5–3 mm, který může opět obsahovat i několik úlomků zeminy či kamínky. S tloušťkou hrany maximálně 1,5 mm a střední tloušťkou maximálně 0,75 mm je vnitřní uzavírací stěna hnízda tenčí než vnější. Ta má na okraji maximálně 2–3 mm a maximálně 1–2,5 mm ve středu, což z ní činí nejsilnější přepážku v hnízdě (Müller & Richter 2019).

Zkoumaná hnízda byla vždy ukryta pod skálou či kameny a i ve svažitém terénu ležela na rovném zemitém povrchu (nikoli na skále). Vzhledem ke špatné přístupnosti některých úkrytů je pravděpodobné, že samice ulity netransportuje, ale vyhledává ty již schované. Důvody hnízdění pod kameny nejsou zcela objasněné, ale důvodem může být ochrana larev před přehřátím, protože teploty půdy na preferovaných stanovištích bývají v důsledku silného slunečního záření vysoké. I když larvy některých Osmiini mají překvapivě vysokou toleranci vůči vysokým teplotám, a navíc bílý povrch ulity by měl riziko přehřátí i na exponovaných místech výrazně snížit. Možná je tedy speciální způsob výběru hnízdiště adaptace na strmé a skalnaté svahy, kdy svažitý terén představuje riziko skutálení ulity a poškození hnízda.

Horizontální plochy pod kameny jsou tedy bezpečnější, navíc je pod nimi velká zásoba prázdných ulit, protože plži se na těchto exponovaných místech pod kameny ukrývají před slunečními paprsky a také zde zimují (Müller & Richter 2019).

*O. andrenoides* přezimuje v hnízdě jako dospělec (Müller & Richter 2019, Müller 2021).

Jedná se o polylektický druh (Westrich 2018, Müller & Richter 2019, Müller 2021). Upřednostňuje čeleď Lamiaceae (Gogala 1999, Westrich 2018, Müller & Richter 2019, Müller 2021), dále sbírá pyl i z čeledí Crassulaceae a Fabaceae (Westrich 2018, Müller 2021), Cistaceae, Brassicaceae, Campanulaceae a Asteraceae (Müller 2021).

Mezi parazitoidy tohoto druhu patří zlatěnky *Chrysis analis* Spinola, 1808, *C. cuprea* a *C. dichroa* (Müller 2021).

### 2.2.3 *Osmia* (*Helicosmia*) *aurulenta* (Panzer, 1799)

Druh preferující travnaté a křovinné (Bogusch a kol. 2020a) skalnaté svahy na vápencovém podloží, například extenzivně využívané i ladem ležící ovčí pastviny, staré vinice, vnitrozemské duny, strukturované okraje lesů, opuštěné lomy, ale i kamenité a řídce zarostlé silniční náspy (Westrich 2018). Vyskytuje se tedy na lokalitách přírodního i antropogenního původu (Heneberg a kol. 2020).

Hnízda si staví v prázdných ulitách plžů různých (středních až velkých) velikostí (Bellmann 1981, Gogala 1999, Müller a kol. 2018, Westrich 2018, Heneberg a kol. 2020, Müller 2021). Osidluje například druhy *H. pomatia*, *Arianta arbustorum* (Linnaeus, 1758) (Bellmann 1981, Westrich 2018), *C. hortensis*, *Caucasotachea vindobonensis* (Férussac, 1821), *X. obvia* (Westrich 2018), *Eobania vermiculata* (O. F. Müller, 1774), *Cornu aspersum* (O. F. Müller, 1774), *Otala lactea* (O. F. Müller, 1774) (Bogusch a kol. 2020b). Preferují větší ulity (Heneberg a kol. 2020) a menší využívají až tehdy, když velké nejsou k dispozici (Bellmann 1981). Existují výzkumy, že samice staví hnízda i v mrtvém dřevě, kolmých písečných svazích, v zemi, ostružiníkových stoncích či skalních štěrbinách, nejsou ale potvrzené (Müller 2021).

V hnízdě bývá vytvořeno několik plodových buněk, v menších ulitách v počtu 1–4, ve větších (např. u *H. pomatia*) 7–12 (Bellmann 1981, Westrich 2018). Nalezeno bylo ale i hnízdo s 17 komůrkami (Bellmann 1981, Müller a kol. 2018, Müller 2021).

Předtím, než začne samice zásobovat první komůrku pylem a nektarem, vystaví v ulitě přepážku a v podobě prstence naznačí další přepážku, čímž vymezí prostor pro první komůrku (Bellmann 1981, Westrich 2018). Materiálem na stavbu hnízda je listová pasta. Během

přinášení rozžvýkaného listového materiálu se samice zastavuje na ulitě a téměř vždy nanese část pasty i na její povrch (Bellmann 1981, Müller a kol. 2018, Westrich 2018, Müller 2021). Ve chvíli, kdy je první přepážka postavena a další naznačena, zásobí samice komůrku pylem a nektarem, naklade vajíčko a prstenec doplní listovou pastou, čímž plodovou buňku uzavře. Následně vytyčí prostor pro další komůrku, opět naznačením další přepážky vystavěním prstence z rostlinné pasty. I nyní část rostlinného materiálu nanese na povrch ulity (Bellmann 1981).

Výsledný tvar hnízda je do značné míry určen tvarem dutiny uvnitř ulity. První dvě buňky jsou prodlouženy, buňky následující se postupně zkracují a rozšiřují. U prvních dvou komůrek jsou tedy zásoby pylu a nektaru umístěny v jejich zadní polovině, u následných kratších a vyšších komůrek v polovině horní (Bellmann 1981, Westrich 2018). U velkých ulit, kde se dutina směrem ven silně rozšiřuje, se samice odchýlí od pravidelného lineárního budování buněk a poslední plodové komůrky postaví vedle sebe (Bellmann 1981, Müller a kol. 2018, Westrich 2018, Müller 2021). Následně vytvoří jednu prázdnou buňku a hnízdo v ústí uzavře pevnou zátkou. U stavby zátky si počíná obdobně jako u stavění přepážek mezi buňkami, nejprve z listové pasty utvoří prstenec, který postupně směrem ke středu uzavírá. U malých ulit může mít výsledná zátka sílu až 5 mm (Bellmann 1981, Westrich 2018). *O. aurulenta* s ulitou nepohybuje (Bellmann 1981, Müller a kol. 2018, Westrich 2018, Müller 2021).

*O. aurulenta* přezimuje v hnízdě jako dospělec (Bellmann 1981, Müller & Richter 2019).

Jedná se o polylektický druh upřednostňující čeleď Fabaceae a Lamiaceae (Bellmann 1981, Westrich 2018, Müller 2021), dále sbírá pyl na čeledích Asteraceae, Boraginaceae, Cistaceae nebo Plantaginaceae (Westrich 2018), Müller (2021) zmiňuje ještě Polygalaceae.

Z blanokřídlého hmyzu v hnízdech parazituje *Chrysura dichroa* (Heneberg a kol. 2020). Bellmann (1981) uvádí ještě *C. trimaculata* (Förster, 1853), Wiesbauer a kol. (2020) zmiňují navíc i *C. cuprea*.

### 2.2.4 *Osmia* (*Hoplosmia*) *spinulosa* (Kirby, 1802)

Druh obývá teplé suché stanoviště na vápenatém podkladu. Vyskytuje se na vnitrozemských dunách, zvětrávajících haldách, v opuštěných lomech, ladem ležících ovčích pastvinách, teplých okrajích lesů, starých vinicích, zřídka i na teplých a suchých ruderálních stanovištích (Westrich 2018).

Hnízdí obligátně v prázdných ulitách plžů (Müller a kol. 2018), vybírá si schránky menších velikostí (Westrich 2018, Müller 2021): *H. itala, X. obvia, Cepaea nemoralis* (Linnaeus, 1758), *Z. detrita*, bylo zaznamenáno i hnízdo v malé ulitě *H. pomatia* (Müller 1994, Westrich 2018), dále v ulitách druhu *Fruticicola fruticum* (O. F. Müller, 1774) (Westrich 2018, Müller 2021), rodů *Cernuella* Schlüter, 1838 a *Pomatias* (Müller 2021) a druhů *Euomphalia strigella* (Draparnaud, 1801) (Bogusch a kol. 2019) a *M. cartusiana* (Bogusch a kol. 2019, 2020a). Heneberg a kol. (2020) tvrzení o výběru menších ulit vyvracejí, v rámci jejich pozorování si *O. spinulosa* vybírala velké ulity *H. pomatia* výrazně častěji než menší ulity *X. obvia.* Müller (1994) uvádí ulity plžů *Z. detrita, F. fruticum, X. obvia, H. itala, Cepaea* sp. a malou ulitu *H. pomatia* s tím, že samice výrazně prerefovaly ulity *F. fruticum* a *X. obvia* před *Z. detrita*.

Počet komůrek v hnízdě se pohybuje mezi jednou a třemi (Müller 1994, 2021, Müller a kol. 2018), většinou samice staví komůrky dvě (Müller 1994, 2021). Plodové buňky jsou uspořádány lineárně (Müller a kol. 2018). Jako hnízdní materiál používá rostlinnou maltu z rozžvýkaných listů (Müller 1994, 2021, Gogala 1999, Müller a kol. 2018, Westrich 2018). Tu používá na výstavbu přepážek, ale nepokrývá jí povrch ulity (Müller 1994, 2021, Müller a kol. 2018). Zahnízděnou ulitu nikam netransportuje, ale otáčí s ní na místě tak, aby její ústí směřovalo k zemi (Müller a kol. 2018, Müller 2021).

Samice před začátkem hnízdění ulitu nečistí. Nebyla zjištěna preference umístění ulit, některá nalezená hnízda byla volně položená a dobře přístupná, jiná byla ukrytá pod vegetací (Müller 1994).

*O. spinulosa* před zásobováním první komůrky nestaví přepážku směrem dovnitř ulity (Müller 1994, 2021). Předtím, než začne zásobovat komůrku pylem a nektarem, naznačí si místo, kde bude přepážka. V hnízdech *O. spinulosa* lze rozlišit tři různé typy stěn, které se liší nejen svou polohou vzhledem k poloze plodových buněk, ale také odlišnou tloušťkou a různě zesílenou okrajovou oblastí. Přepážka za první (vnitřní) komůrkou je velmi tenká (0,15–0,3 mm uprostřed, po okrajích mírně zesílená na 0,3–0,6 mm) a není dokonale přilnutá ke stěně ulity. U hnízd s jedinou plodovou komůrkou se tato tenká přepážka nevyskytuje, v hnízdech se třemi plodovými komůrkami jsou takovéto tenké přepážky dvě. Vnější přepážka uzavírající poslední buňku je naopak nápadně silná (0,3–0,5 mm uprostřed a 0,8–2 mm při okrajích) a ke stěnám ulity je přilnutá velmi dobře. Tato přepážka je v hnízdě *O. spinulosa* vždy jen jedna. Zátka má ve středu stejnou tloušťku jako předešlá přepážka (0,3–0,5 mm), ale po okrajích není tak zesílená (0,5–0,9 mm). Je vždy v oblasti ústí (Müller 1994). Silná vnější přepážka společně se zátkou slouží jako obrana proti parazitům (Müller 1994, 2021). Prostor mezi vnější přepážkou

a zátkou je prázdný, ničím nevyplněný. Po dokončení zátky samice ulitu otočí ústím dolů, tím je proces stavby hnízda u konce a samice začne hledat novou prázdnou ulitu. Otočená ulita již není zakryta žádným materiálem (Müller 1994).

Postupným vysycháním materiálu dochází u zátky k vytváření mezer v místech styku s ulitou, což může vést až k vypadnutí zátky a ztrátě její ochranné funkce. Je možné, že otočení ulity ústím dolů proces vysychání zpomalí. Samice *O. spinulosa* se k (alespoň některým) svým již hotovým hnízdům vracejí a v případě potřeby opravují otvory a štěrbiny v zátkách nově přinesenou rostlinnou maltou (Müller 1994).

*O. spinulosa* je letní druh (Müller & Richter 2019, Hostinská a kol. 2021), s dospělci se můžeme setkat od června do srpna (Gogala 1999, Westrich 2018). Zimu přečkává v hnízdech jako předkukla a svůj vývoj dokončuje až po přezimování (Müller 1994, Westrich 2018, Müller & Richter 2019, Hostinská a kol. 2021). Ne všechny larvy se vyvinou v dospělce už po prvním roce hibernace, určité procento ve stádiu předkukly přezimuje dvakrát a metamorfózu prodělává až poté (Müller 1994).

Jedná se o oligolektický druh specializující se na čeleď Asteraceae (Müller 1994, 2021, Gogala 1999, Westrich 2018).

Z blanokřídlého hmyzu v hnízdech parazituje kukaččí včela *S. odontopyga* (Müller 1994, Kasparek 2015, Westrich 2018, Bogusch a kol. 2020a, Heneberg a kol. 2020), chalcidky *Pteromalus venustus* Statz, 1938 a *Melittobia acasta* (Walker, 1839) (Müller 1994) a zlatěnky *C. cuprea* (Müller 1994, Heneberg a kol. 2020), *C. trimaculata* a *C. dichroa* (Müller 1994, Wiesbauer a kol. 2020).

### 2.2.5 *Osmia* (*Neosmia*) *bicolor* (Schrank, 1781)

Druh se vyskytuje na okrajích lesů, skalnatých svazích, vřesovištích, travních porostech na bazickém podloží, ve starých vinicích, ale také na kamenitých náspech silnic (Westrich 2018). Obývá travnaté a křovinaté svahy (Bogusch a kol. 2020a), vyskytuje se na lokalitách přírodních i antropogenních, přičemž ty přírodní silně preferuje (Bogusch a kol. 2019, Heneberg a kol. 2020).

Hnízdí obligátně v prázdných ulitách plžů (Müller a kol. 2018). K hnízdění si vybírá ulity *F. fruticum, C. vindobonensis* (Westrich 2018, Heneberg a kol. 2020), *C. nemoralis, C. hortensis, A. arbustorum* (Westrich 2018), *H. pomatia* (Westrich 2018, Bogusch a kol. 2019), *Aegopis verticillus* (Lamarck, 1822) (Bogusch a kol. 2019). Obvykle preferuje ulity

střední velikosti (např. *Cepaea* sp.), velké ulity hlemýžďů si vybírá méně často (Bellmann 1981, Müller 2021).

Jakmile samice nalezne vhodnou ulitu, zkontroluje její vnitřek. Pokud je ústí natočené směrem vzhůru, otáčí s ulitou tak, aby ho nasměrovala dolů. Aby mohla samice s ulitou pohnout, zapře se mezi ulitu a vegetaci a schránku otočí pomocí nohou (Bellmann 1981, Westrich 2018).

Následně samice odlétá k okolním rostlinám, ukousne malé kousky listu, které zpracuje za pomoci kusadel a slin do kašovité hmoty, kterou přenáší do ulity (Bellmann 1981, Westrich 2018). Z této hmoty pak zhotovuje hnízdní přepážky, používá ji i ke stavbě zátky a nanáší ji taktéž na povrch ulity (Bellmann 1981, Müller a kol. 2018, Westrich 2018, Müller 2021). Během stavby hnízda samice občas ulitu pootočí do jiné polohy (Bellmann 1981).

Hnízda obsahují obvykle jen jednu komůrku (Bellmann 1981, Müller 2021), někdy se ale (např. v ulitě *H. pomatia*) mohou vyskytnout až čtyři (Westrich 2018) nebo pět (Müller a kol. 2018, Müller 2021), ty jsou pak postaveny lineárně za sebou (Müller a kol. 2018). Tvar hnízdní komůrky je dán tvarem ulity. Přepážka před první komůrkou směrem dovnitř ulity není postavena. Za nashromážděnou směsí pylu a nektaru je prostor o délce přibližně 1 cm, za kterým následuje přepážka z rostlinné pasty. Tato přepážka je u poslední komůrky součástí zátky (Bellmann 1981).

Zátka uzavírající poslední komůrku a zároveň celé hnízdo je široká 1–2 cm (Bellmann 1981, Müller 2021) a sestává ze dvou přepážek z rostlinné pasty a prostoru mezi nimi vyplněném malými kamínky, úlomky zeminy, rozlámanými kousky ulit a kousky dřeva (Bellmann 1981, Müller a kol. 2018, Westrich 2018, Müller 2021). Těchto komůrek vyplněných kamínky a dalším materiálem a oddělených od sebe přepážkami z rostlinné pasty může být v ulitě více (Bellmann 1981, Westrich 2018). Silná zátka slouží jako bariéra proti parazitům (Müller 1994).

Jak již bylo zmíněno, samice s ulitou před hnízděním i během něho pohybuje (Bellmann 1981, Müller 1994). K manipulaci s ulitou dochází i po dokončení hnízda – zahnízděná ulita je otočena tak, aby její ústí směřovalo k zemi (Bellmann 1981, Westrich 2018, Müller 2021). Příležitostně samice ulitu zakope 1,5 cm hluboko do země (Müller 2021). Bellmann (1981) a Westrich (2018) uvádějí, že včela vyhrabává jamku tehdy, pokud se jí nedaří nasměrovat ústí k zemi pouhým otočením ulity. V tom případě hloubí díru pomocí mandibul a uvolněnou zeminu odnáší za letu cca 5–10 cm daleko (Bellmann 1981, Westrich 2018). Pokud leží ulita na mechu nebo v trávě, snaží se samice co nejvíce tohoto rostlinného materiálu odkousat, opět z důvodu snadnějšího otočení ulitou (Westrich 2018). Po otočení ulity a případném zakopání je

hnízdo zakryto stovkami suchých stébel nebo borových jehlic (Bellmann 1981, Müller 1994, 2021, Westrich 2018). Amiet (1973) uvádí, že pokud je skrýš (experimentálně) odstraněna, samice materiál nanosí znovu, což naznačuje, že *O. bicolor* kontroluje i svá již dokončená hnízda.

Zimu přečkává jako imago uvnitř hnízda (Bellmann 1981, Westrich 2018, Müller & Richter 2019).

Jedná se o polylektický druh sbírající pyl na rostlinách z 13 čeledí – Apiaceae, Asparagaceae, Asteraceae, Boraginaceae, Brassicaceae, Cistaceae, Fabaceae, Lamiaceae, Ranunculaceae, Rosaceae, Salicaceae, Saxifragaceae, Violaceae (Westrich 2018). Müller (2021) uvádí dokonce 18 čeledí.

Podle Westricha (2018) nebyli dosavadními výzkumy prokázáni žádní hnízdní paraziti. Jako parazitoidi se zde vyskytují drvenka *S. quinquepunctata* (Bogusch a kol. 2020c) a zlatěnky *Chrysura dichroa* (Heneberg a kol. 2020), *C. trimaculata* (Bellmann 1981, Wiesbauer a kol. 2020), *C. refulgens* (Spinola, 1806) (Strumia 1997), *C. austriaca* (Fabricius, 1804) a *C. cuprea* (Wiesbauer a kol. 2020).

### 2.2.6 *Rhodanthidium* (*Rhodanthidium*) *septemdentatum* (Latreille, 1809)

Druh upřednostňuje stanoviště stepního charakteru (Hostinská a kol. 2021), tedy místa suchá a teplá (např. suché pastviny a skalnaté stepi) (Kasparek 2019).

Hnízdí v prázdných ulitách plžů, např. *Cepaea* sp., *E. vermiculata*, *C. aspersum* (Kasparek 2019, Hostinská a kol. 2021), *Levantina bellardi* (Mousson, 1854), *T. pisana*, *Helix lucorum* Linnaeus, 1758 (Kasparek 2019), *Sphincterochila candidissima* (Draparnaud, 1801) nebo *Cernuella virgata* (da Costa, 1778) (Hostinská a kol. 2021).

V hnízdě se nachází 1–2 hnízdní komůrky (Pasteels 1977, Kasparek 2019, Hostinská a kol. 2021).

Materiálem na stavbu hnízda je pryskyřice (Pasteels 1977, Kasparek 2019, Hostinská a kol. 2021). Hnízdo je tvořeno hnízdní komůrkou s pylem, za kterou následuje pryskyřičná přepážka, komůrka volně vyplněná kamínky, pískem a suchým rostlinným materiálem a zátka z fragmentů ulit slepených pryskyřicí (Grandi 1961, Hostinská a kol. 2021). Vnitřní přepážka před první komůrkou není vytvořena (Hostinská a kol. 2021).

Hnízdí v ulitách pod kameny (Pasteels 1977, Kasparek 2019, Hostinská a kol. 2021) nebo ukrytých v kamenných zídkách (Hostinská a kol. 2021). Zahnízděná ulita je dle Pasteelse (1977) a Kasparka (2019) otočena ústím dolů, během našich výzkumů (Hostinská a kol. 2021)

jsme ovšem žádnou manipulaci s ulitou nezaznamenali. Samice si vybírá ulity již ukryté (Hostinská a kol. 2021).

Přezimuje jako dospělec uvnitř hnízda (Hostinská a kol. 2021).

Jedná se o polylektický druh, který sbírá pyl z mnoha čeledí rostlin (Kasparek 2019, Hostinská a kol. 2021), např. Lamiaceae (Kasparek 2019), Fabaceae (Kasparek 2019, Hostinská a kol. 2021), Boraginaceae, Rosaceae, Fagaceae, Plantaginaceae (Hostinská a kol. 2021). Müller (1996) uvádí až 17 čeledí.

V hnízdech parazituje kukaččí včela *Stelis ruficornis* Morawitz, 1872 a zlatěnka *C. refulgens* (Kasparek 2019).

## 2.3 Paraziti v ulitách

V hnízdech samotářských včel se vyskytují také nejrůznější blanokřídlí paraziti, kteří si nestaví vlastní hnízda, ale jejich larvy se vyvíjejí na úkor svého hostitele. Dle zdroje potravy zde rozlišujeme kleptoparazity (hnízdní paraziti, kukaččí včely) a parazitoidy (Macek a kol. 2010).

Kleptoparazitismus (hnízdní parazitismus) je speciální forma parazitismu, kdy se potomstvo parazita vyvíjí na potravních zásobách nashromážděných původně pro hostitelskou larvu (Litman 2019). Samice kukaččí včely tedy klade vajíčka na pylonektarové zásoby, které hostitelská samice nasbírala pro své vlastní potomstvo (Michener 2007, Litman 2019). Potravní zásoby však nejsou dostatečné k tomu, aby se na nich mohly vyvíjet obě larvy (hostitele i parazita) společně, potomstvo hostitele je proto parazitem odstraněno (Litman 2019).

Samice parazitoidů kladou vajíčka na vhodného hostitele nebo do jeho blízkosti, vylíhlé larvy během svého vývoje hostitele postupně konzumují, až ho nakonec usmrtí. Tato životní strategie se vyskytuje u mnoha skupin hmyzu (např. Diptera a Coleoptera), největší diverzita parazitoidů však byla popsána u řádu Hymenoptera (Quicke 1997, Henri & Van Veen 2011).

U včel hnízdících v ulitách se můžeme setkat jak s kukaččími včelami, tak s parazitoidy (Bellmann 1981, Müller 1994, 2021, Strumia 1997, Westrich 2018, Kasparek 2019, Bogusch a kol. 2020b, 2020c, Heneberg a kol. 2020, Hostinská a kol. 2021). V ČR parazitují u ulitových včel především kukaččí včela *S. odontopyga*, zlatěnky *C. cuprea, C. dichroa* a *C. trimaculata* (Bogusch a kol. 2020c) a drvenka *S. quinquepunctata* (Bogusch a kol. 2020b).

### 2.3.1 *Stelis odontopyga* Noskiewicz, 1926

*S. odontopyga* je xerotermofilní druh včely z čeledi Megachilidae vyskytující se na skalních stepích (Macek a kol. 2010). Je to hnízdní parazit včel druhu *O. spinulosa* (Blüthgen 1926, Müller 1994, Gogala 1999, Kasparek 2015, Westrich 2018, Edwards a kol. 2019, Bogusch a kol. 2020a, 2020c, Heneberg a kol. 2020). Dospělci se živí nektarem například na čeledích Apiaceae, Asteraceae, Boraginaceae (Kasparek 2015, Edwards a kol. 2019).

### 2.3.2 Zlatěnkovití (Chrysididae)

Zlatěnky jsou kovově leskle zbarvení ektoparazitoidi, jejichž larvy se živí masožravě, u včel hnízdících v ulitách je jejich potravou samotná včelí larva (Wiesbauer a kol. 2020, Agnoli & Rosa 2022). Dospělci se živí nektarem (Westrich 2018). Pro vykladení vlastního vajíčka musí samice zlatěnky zvolit vhodný okamžik, proto hlídkuje v okolí hnízda a občas ho kontroluje (Macek a kol. 2010). Pokud se stane, že je samice zlatěnky v hnízdě přistižena, vtáhne končetiny do prohlubně zadečku a stočí se do kuličky, což jí poskytne ochranu před útokem hostitelské samice. Samice zlatěnky obvykle klade vajíčko (někdy dvě) na vnitřní stranu připravené koncové stěny plodové buňky, někdy na zásoby pylu (Westrich 2018). Larva zlatěnky se po vylíhnutí přesune na larvu včely, zpočátku ale svého hostitele jen krátce nasává a poškodí ho jen neznatelně. Teprve když larva hostitele vyroste a začne spřádat kokon, zlatěnka ji úplně vysaje a poté se zakuklí (Westrich 2018, Wiesbauer a kol. 2020).

*Chrysura cuprea* je xerotermofilní druh obývající stepní biotopy a sprašové stěny (Macek a kol. 2010). Parazituje u všech druhů našich včel hnízdících v ulitách s výjimkou druhu *R. septemdentatum* (Wiesbauer a kol. 2020, Agnoli & Rosa 2022). Nesoustředí se pouze na hnízda v ulitách, vajíčka klade i do hnízd jiných samotářských včel, preferuje především rody *Osmia*, *Hoplitis*, *Anthocopa* Lepeletier & Serville, 1825, *Chelostoma* Latreille, 1809 a *Chalicodoma* Lepeletier, 1841 (Macek a kol. 2010).

*Chrysura dichroa* obývá zejména skalní stepi a parazituje především u včel hnízdících v ulitách (Macek a kol. 2010). Byla zaznamenána u druhů *O. andrenoides*, *O. aurulenta*, *O. rufohirta* a *O. spinulosa* (Wiesbauer a kol. 2020), Heneberg a kol. (2020) ji zmiňují i u druhu *O. bicolor*.

*Chrysura trimaculata* se vyskytuje na exponovaných stepích a jejími hostiteli jsou včely stavící si svá hnízda v prázdných ulitách (Macek a kol. 2010). Je parazitoidem druhů *O. aurulenta*, *O. bicolor* a *O. spinulosa* (Wiesbauer a kol. 2020, Agnoli & Rosa 2022).

Během našich výzkumů v České republice jsme v ulitách zaznamenali navíc ještě druh *Hedychrum niemelai* Linsenmaier, 1959 v ulitě rodu *Cepaea*, ovšem bez hnízda (Heneberg a kol. 2020), v zahraničí pak například druh *Chrysura hybrida* (Lepeletier, 1806) v hnízdech *H. fertoni*. Jedná se o nejběžnějšího parazitoida, který se specializuje na menší druhy náležící do tribu Osmiini bez ohledu na jejich hnízdní substrát (Bogusch a kol. 2020c). V hnízdech *H. fertoni* ji zaznamenal již Le Goff (2003). Dále jsme během zahraničních výzkumů objevili druhy *C. refulgens* (v hnízdech *R. sticticum*) (Hostinská a kol. 2021) a *C. rufiventris* (Dahlbom, 1854) (Bogusch a kol. 2020b).

### 2.3.3 *Sapyga quinquepunctata* (Fabricius, 1781)

Dalším blanokřídlým parazitem, který byl objeven v hnízdech ulitových včel, je drvenka *S. quinquepunctata* (Sapygidae, Vespoidea) (Bogusch a kol. 2020b). Preferuje otevřené biotopy (Bees, Wasps & Ants Recording Society 2022a), v ČR se vyskytuje převážně na slunných stanovištích (Macek a kol. 2010). Její hostitelé hnízdí v různých substrátech, například v jílu, otvorech ve zdech, mrtvém dřevě či prázdných ulitách plžů (Bees, Wasps & Ants Recording Society 2022a). Samice kladou vajíčka do hnízd v době nepřítomnosti hostitelské samice (Bogusch a kol. 2007). Parazituje u včel rodů *Osmia*, *Chelostoma* (Bogusch a kol. 2007, Bees, Wasps & Ants Recording Society 2022a), *Hoplitis* a *Megachile* Latreille, 1802 (Bogusch a kol. 2007). V ulitách byla v rámci našich výzkumů nalezena u druhu *O. bicolor* (Bogusch a kol. 2020b) a *O. ferruginea* (Bogusch a kol. 2020c).

Životní strategie druhu *S. quinquepunctata* i celé čeledi Sapygidae není zcela jasná. Dospělci čeledi Sapygidae se živí pylem a nektarem (Bogusch a kol. 2007), názory na potravu larev se však různí (Gusenleitner & Gusenleitner 1994, O'Neill 2001, Bogusch a kol. 2007, Macek a kol. 2010, Schmid-Egger 2010, Bees, Wasps & Ants Recording Society 2022a).

Gusenleitner & Gusenleitner (1994) a Bogusch a kol. (2007) druhy čeledi Sapygidae řadí mezi zástupce kleptoparazitů. Samice údajně kladou vajíčka do plodových komůrek hnízd jiných samotářských včel, dříve vylíhlá larva parazita následně odstraní potomstvo hostitele (Bogusch a kol. 2007). Larva prvního instaru druhu *S. quinquepunctata* má vyvinuté velké mandibuly a zničené vajíčko hostitele pozře. Larvy dalších instarů mají již kusadla menší a živí se nahromaděnými pylonektarovými zásobami (Bees, Wasps & Ants Recording Society 2022a).

Schmid-Egger (2010) uvádí, že samice zástupců čeledi Sapygidae kladou vajíčka do hnízd samotářských včel, především z čeledi Megachilidae, a vyvíjející se parazitická larva se živí jak hostitelskou larvou, tak nahromaděnými zásobami potravy. Macek a kol. (2010) předkládají podobné informace. Dle nich zástupci čeledi Sapygidae preferují čeleď Megachilidae a jedná se buď o kleptoparazity živící se pylonektarovými zásobami, nebo o ektoparazitoidy, kteří se vyvíjí na dospělých larvách nebo předkuklách, přičemž životní strategie je u různých druhů různá. Druh *S. quinquepunctata* je zde uveden jako parazitoid (Macek a kol. 2010). Dle O'Neilla (2001) jsou taktéž zástupci čeledi Sapygidae ektoparazitoidi i kleptoparaziti.

### 2.3.4 Jiní paraziti ulitových včel

V hnízdech ulitových včel mohou parazitovat i další zástupci blanokřídlého hmyzu, například chalcidky *Pteromalus venustus* a *Melittobia acasta* (Müller 1994).

*P. venustus* je ektoparazitoid předkukel a kukel včel čeledi Megachilidae. Samice kladélkem propichuje hostitelské kokony v hnízdech, přičemž na jednoho hostitele klade více (někdy i přes 30) vajíček (Tepedino 1993). Müller (1994) tento druh objevil v hnízdech *O. spinulosa*.

Chalcidky rodu *Melittobia* Westwood, 1847 jsou ektoparazitoidi vos a včel, a to především těch, které si staví hnízda z jílu. *M. acasta* je jediným evropským druhem tohoto rodu a je uváděna jako parazit a hyperparazit u zástupců různých řádů (např. Hymenoptera, Diptera, Lepidoptera a Coleoptera). U blanokřídlého hmyzu tvoří její hostitelskou skupinu sociální (např. *Bombus* Latreille, 1802, *Apis* Linnaeus 1758) i samotářské včely (*Anthophora* Latreille, 1803, *Chalicodoma*, *Heriades* Spinola, 1808, *Anthidium* Fabricius, 1804, *Megachile*, *Osmia*) a jejich paraziti (*Psithyrus* Lepeletier, 1833, *Stelis* Panzer, 1806) a dále také samotářské i sociální vosy či kutilky (González a kol. 2004). U ulitových včel byla nalezena v hnízdě *O. spinulosa* (Müller 1994).

V rámci našich výzkumů jsme v ulitách objevili také kodulky *Stenomutilla collaris* (Fabricius, 1787) a *S. hotentotta* (Fabricius, 1804) a kukaččí včelu *Dioxys moesta* Costa, 1883 (Bogusch a kol. 2020c).

Kodulky (Mutillidae) jsou ektoparazitoidi s obvykle širokým spektrem hostitelů, často z řádu blanokřídlého hmyzu. Biologie zástupců rodu *Stenomutilla* André, 1896 není příliš prozkoumaná. Je však pravděpodobné, že druhy *S. collaris* a *S. hotentotta* parazitují u různých druhů včel a možná i vos. Během našich výzkumů jsme je zaznamenali ve Španělsku v hnízdech *H. fertoni* (Bogusch a kol. 2020c). U ulitových včel mohou parazitovat také kodulky rodu *Tricholabiodes* Radoszkowski, 1885 (Gess & Gess 2008, Heneberg a kol. 2020).

Evropské druhy rodu *Dioxys* Lepeletier & Serville, 1825 jsou kleptoparaziti včel čeledi Megachilidae, většinou rodů *Hoplitis* a *Osmia*, ovšem dosud nebyly zaznamenány u druhů hnízdících v ulitách (Westrich 2018, Bogusch a kol. 2020c). *D. moesta* je kukaččí včela s neznámým zaměřením na hostitele, během našich výzkumů byla nalezena v hnízdě *H. fertoni* (Bogusch a kol. 2020c).

V hnízdech ulitových včel parazitují i zástupci z jiných řádů, např. Diptera nebo Coleoptera (Heneberg a kol. 2020). Během našich výzkumů jsme zde zaznamenali parazitickou mouchu *Anthrax aethiops* (Fabricius, 1781) (Diptera: Anthracidae) a brouka *Trichodes apiarius*

(Linnaeus, 1758) (Coleoptera: Cleridae). V obou případech se jedná o běžné parazitoidy ulitových včel (Bogusch a kol. 2020b).

Druh *A. aethiops* parazituje především u včel čeledi Megachilidae, je ale možné, že klade vajíčka i do hnízd včel jiných čeledí (Bogusch a kol. 2020c). V ulitových hnízdech byl objeven u druhů *O. spinulosa* (Müller 1994, Heneberg a kol. 2020), *O. aurulenta, R. septemdentatum* (Čelechovský 2015), ve Španělsku jsme ho nalezli i u druhů *H. fertoni* a *O. ferruginea* (Bogusch a kol. 2020c).

Larvy brouka druhu *T. apiarius* se vyvíjí u samotářských včel, které hnízdí například na místech s obnaženou půdou, sprašových a pískových stěnách či ve zdech, příležitostně se nalézají i ve včelích úlech. Dospělci se vyskytují na květech především čeledi Apiaceae, kde se živí pylem, ale také loví jiný hmyz (Škorpík 2018).

## 2.4 Další blanokřídlí hnízdící v ulitách

V prázdých ulitách plžů si nestaví hnízda pouze samotářské včely, ale i další blanokřídlí, například některé druhy čeledí Pompilidae (Richards & Hamm 1939), Vespidae (Fateryga 2013) či Formicidae (Bogusch a kol. 2019, Bees, Wasps & Ants Recording Society 2022b). Následující podkapitoly se zaměřují především na druhy vyskytující se na území České republiky.

### 2.4.1 Hrabalkovití (Pompilidae)

Zástupci čeledi Pompilidae mají hnízdní strategie různé. Může se jednat buď o ektoparazitoidy, predátory, či hnízdní parazity u jiných druhů hrabalek. Larvy jsou masožravé a jejich potravou jsou výhradně pavouci (Bogusch a kol. 2007). Dospělci se živí především květním nektarem, samice však příležitostně sají i hemolymfu z ran ulovených pavouků (Macek a kol. 2010). Mezi predátory hnízdící v ulitách patří například hrabalky *Anoplius nigerrimus* (Scopoli, 1763), *Auplopus carbonarius* (Scopoli, 1763), *Agenioideus cinctellus* (Spinola, 1808), *Homonotus sanguinolentus* (Fabricius, 1793) (Richards & Hamm 1939, Day 1988), *Dipogon variegatus* (Linnaeus, 1758) (Day 1988, Bees, Wasps & Ants Recording Society 2022c) nebo *Priocnemis propinqua* Lepeletier, 1847 (Bogusch a kol. 2020b). Všechny tyto druhy náleží i do fauny České republiky (Bogusch a kol. 2007), pouze *P. propinqua* je na tomto území již vyhynulý (Bogusch & Straka 2017).

*Anoplius nigerrimus* je druh vyskytující se na suchých pastvinách a křovinách (Bees, Wasps & Ants Recording Society 2022d), ale i na březích, vřesovištích a mokřadech (Richards & Hamm 1939). Hnízda si staví na různých substrátech, například pod kameny, v dutých stoncích rostlin, opuštěných chodbách jiných blanokřídlých či v prázdných ulitách plžů (Richards & Hamm 1939, Day 1988). V drobivé půdě je tento druh schopný vykopat si svoji vlastní chodbu (Bees, Wasps & Ants Recording Society 2022d). Richards & Hamm (1939) zmiňují výskyt v ulitě druhu *C. aspersum*, v rámci našich výzkumů jsme tento druh hrabalky objevili na antropogenním stanovišti v ulitě druhu *H. pomatia* (Bogusch a kol. 2019).

*Auplopus carbonarius* je druh obývající vlhké lesy s bažinatými oblastmi, které poskytují vlhký jíl jakožto hnízdní materiál (Bees, Wasps & Ants Recording Society 2022e). Hnízda si staví na různých podkladech, například v kamenných zdech, pod kameny, na pařezech, často také ve starých broučích chodbách, pod kůrou, ve štěrbinách kmenů stromů,

v hálkách druhů *Andricus hungaricus* (Hartig, 1843) a *A. kollari* (Hartig, 1843), v různých dutinách v zemi, ve starých ulitách, včelích úlech a na dalších místech (Richards & Hamm 1939). Mohou se vyskytovat i v blízkosti hospodářských stavení a lidských obydlí (Day 1988). Plodové buňky vystavěné z jílu mají soudkovitý tvar s plochými konci, jsou položeny na boku a navzájem se dotýkají, přičemž jejich uspořádání může být lehce nepravidelné (Richards & Hamm 1939, Day 1988). Jednotlivé buňky mohou být vystavěny z jílových pelet různého původu, a tedy mohou mít různou barvu a texturu. Vnitřek komůrek je vyhlazený (Day 1988). Jejich počet je variabilní (zaznamenáno bylo od 2 do 34). Někdy si staví hnízdo několik samic *A. carbonarius* pohromadě, krom toho mohou být hnízda vystavěná v blízkosti hnízd jiných blanokřídlých, např. hrnčířky *Ancistrocerus oviventris* Wesmael, 1836, dále byla nalezena i ve starých hnízdech pelonosky rodu *Anthophora* (Richards & Hamm 1939). V rámci našich výzkumů jsme tento druh hrabalky objevili na antropogenních i přírodních stanovištích v ulitách druhu *H. pomatia* (Bogusch a kol. 2019).

*Agenioideus cinctellus* je druh vyskytující se na sušších stanovištích a písčitých půdách. K hnízdění si vybírá nejrůznější přírodní dutiny, případně také chátrající zdi (Day 1988). Využívá i stará hnízda jiných blanokřídlých (např. *Hoplitis adunca* (Panzer, 1798), *Colletes* Latreille, 1802, *Odynerus* Latreille, 1802), dále hnízdí ve ztrouchnivělém dřevě a ulitách. Vzácně si zřejmě hloubí své vlastní dutiny (Richards & Hamm 1939). Plodové buňky uzavírá detritem (Day 1988).

Druh *Homonotus sanguinolentus* byl zaznamenán například na písečných vřesovištích (Day 1988). Napadá zápřednice *Cheiracanthium erraticum* (Walckenaer, 1802) přímo v jejich hnízdech, která jsou stavěna například ve svinutých listech rostlin. Existuje záznam nálezu hnízda v ulitě *C. aspersum*, kdy byl vstup do hnízda ucpán fragmenty rostlin a zeminy (Richards & Hamm 1939).

Druh *Dipogon variegatus* se vyskytuje v široké škále otevřených biotopů jako jsou parky, vřesoviště, pobřežní lokality nebo předměstské zahrady (Bees, Wasps & Ants Recording Society 2022c). Hnízdí v nejrůznějších dutinách ve dřevě, zdech a také v prázdných ulitách plžů. Také bylo pozorováno hloubení vlastní dutiny v měkké maltě pomocí kusadel. Po zásobení potravou je plodová buňka uzavřena materiálem jako jsou zrnka písku, úlomky zeminy a fragmenty rostlinného materiálu údajně spojených dohromady pavučinou (Day 1988).

*Priocnemis propinqua* je pravděpodobně druhem písčitých půd. Jeho hnízdní biologie není známá (Bees, Wasps & Ants Recording Society 2022f), při našich výzkumech byl poprvé objeven v prázdných ulitách plžů, a to konkrétně v druzích *O. lactea* a *S. candidissima* (Bogusch a kol. 2020b).

### 2.4.2 Sršňovití (Vespidae)

Z čeledi Vespidae bylo hnízdění v ulitách zaznamenáno např. u tří palearktických druhů: *Leptochilus alpestris* (Saussure, 1856), *L. mauritanicus* (Lepeletier, 1841) a *L. regulus* (Saussure, 1856) (Fateryga 2014), přičemž v ČR se vyskytují dva z nich – hrnčířky *L. alpestris* a *L. regulus* (Bogusch a kol. 2007).

V případě hrnčířky *Leptochilus alpestris* se pravděpodobně jedná o druh hnízdící výhradně v prázdných ulitách plžů, hnízda byla nalezena ve schránkách druhů *C. nemoralis, Candidula unifasciata* (Poiret, 1801), *Z. detrita, Xeropicta derbentina* (Krynicki, 1836) a *Monacha fruticola* (Krynicki, 1833) (Fateryga 2013). Ve starší literatuře byla hnízda tohoto druhu popsána pouze okrajově, Fabre (1993) uvádí dvě až čtyři plodové buňky na hnízdo a zátku vytvořenou z písku slepeného pryskyřicí. Fateryga (2013) popsal stavbu na základě nálezu jediné zahnízděné ulity od druhu *M. fruticola*. Toto hnízdo bylo nalezeno na skalnatém stepním svahu pod kamenem a obsahovalo jednu plodovou buňku, která byla uzavřena zátkou vyrobenou z (neznámou hmotou) slepených kousků štěrku. Onou lepivou hmotou mohla být pryskyřice, nebo také sliny (Fateryga 2013). My jsme tento druh objevili během našich výzkumů ve Španělsku v ulitách druhů *C. virgata, E. vermiculata* a *S. candidissima* (Bogusch a kol. 2020b).

Hnízdo *Leptochilus mauritanicus* bylo nalezeno v ulitě *S. candidissima* (Ferton 1901, Fateryga 2013, 2014). Přepážky a zátka byly vytvořeny z úlomků ulit smísených s jílem (Ferton 1901, Fateryga 2013). Dle dosud dostupných údajů se jedná o obligátní způsob hnízdění, nálezů bylo však doposud jen velmi málo a neexistují tedy žádné důkazy ohledně toho, že by druh nemohl zahnízdit i v jiném substrátu (Fateryga 2014).

*Leptochilus regulus* je jediný palearktický druh rodu *Leptochilus*, u kterého je známo hnízdění v různých typech již existujících dutin (např. v dutých stoncích, dutinách v kamenech nebo v prázdných ulitách plžů rodu *Helix* Linnaeus, 1758). V ulitách tedy, na rozdíl od předchozích dvou jmenovaných druhů, hnízdí pouze fakultativně. Hnízda obsahují více buněk, přepážky mezi nimi jsou postaveny ze štěrku a zeminy (Fateryga 2014).

### 2.4.3 Mravencovití (Formicidae)

U čeledi Formicidae si ulity jako vhodné dutiny k hnízdění vybírají například druhy *Myrmica sabuleti* Meinert, 1861, *Temnothorax crassispinus* (Karavaiev, 1926), *T. nigriceps* (Mayr, 1855), *T. parvulus* (Schenck, 1852), *T. unifasciatus* (Latreille, 1798) (Bogusch a kol.

2019) či *T. albipennis* (Curtis, 1854) (Bees, Wasps & Ants Recording Society 2022b). Zástupci této čeledi k hnízdění využívají nejrůznější dutiny (např. Seifert 2017), v případě ulit se tedy jedná o hnízdní zdroj fakultativní. Až na posledně jmenovaný druh se jedná o druhy fauny České republiky (Bogusch a kol. 2007).

# 3 DISKUZE

Naše výzkumy lze rozdělit do tří hlavních kategorií – sběr a výzkum samotných ulit, studium hnízdní biologie vybraných druhů včel hnízdících v ulitách a studium změn rozšíření zvolených stepních druhů včel.

Výzkum ulit proběhl nejprve ve střední Evropě a využili jsme k němu dvě metody. Prvním krokem byl sběr ulit na vybraných lokalitách během zimního období a jejich ponechání v laboratoři k probuzení zazimovaných a vylíhnutí uvnitř zahnízděných druhů (Bogusch a kol. 2019). Následovalo dodání označených ulit na lokality, ponechání na místě přes hnízdní sezónu a jejich opětovný sběr (Heneberg a kol. 2020).

Studium prázdných ulit plžů v obdobném rozsahu doposud neproběhlo, v našem případě se tedy jedná o první ucelené výzkumy toho, kteří bezobratlí živočichové (především hmyz a pavouci) ulity využívají. Zjistili jsme, že přítomnost prázdných ulit na antropogenních stanovištích umožňuje přítomnost mnoha druhů hmyzu, které ulity využívají, ovšem zároveň se ukázalo, že samotný výskyt ulit k rozšíření veškerého ulity využívajícího hmyzu z přírodních na antropogenní stanoviště nestačí. Z našich výsledků dále plyne, že i v případě kolonizace těchto nových biotopů zde některé druhy nedosahují stejné početnosti. Důvodem je například nedostatek potravních zdrojů (Bogusch a kol. 2019). Nižší početnost a druhová pestrost různých hmyzích společenstev na antropogenních lokalitách již byla dříve zmiňována více autory (Pereira a kol. 2012, Cardinale 2014, Geslin a kol. 2016, Pereira-Peixoto a kol. 2016). Existují však i výzkumy s opačným výsledkem, zde se ale jedná zejména o druhy (např. žahadlových blanokřídlých) závislé na určitém stupni disturbance prostředí (Cizek a kol. 2013, Tropek a kol. 2013, Heneberg a kol. 2014, Bogusch a kol. 2016).

Naše výzkumy dále poskytly první experimentální důkaz o důležitosti vysoké kvality ulit jako dílčích biotopů, které umožňují přítomnost specializovaných žahadlových blanokřídlých na lokalitě. Úplná absence prázdných schránek plžů na lokalitě zcela brání přítomnosti hmyzu využívajícího ulity, a to i na místech s početnými potravními zdroji. Naopak jejich dodání může zvýšit populace specializovaných žahadlových blanokřídlých dokonce i na místech, kde se prázdné ulity již přirozeně vyskytují. Z hlediska ochrany přírody (a konkrétně ochrany specializovaných žahadlových blanokřídlých) by tedy hlavním opatřením měla být podpora místních populací plžů přísnější ochranou neorné plochy s volnou vegetací, typicky na jižně exponovaných svazích nebo na půdách s nízkou schopností zadržování vody. Přísnější

ochrana takových oblastí by podpořila přítomnost vitálních populací plžů, které generují dostatečné množství prázdných ulit (Heneberg a kol. 2020).

K obdobným poznatkům jsme dospěli i během výzkumů prázdných ulit, které jsme provedli ve Španělsku (Bogusch a kol. 2020b). Výskyt žahadlových blanokřídlých na lokalitách závisí na přítomnosti jejich potravních a hnízdních zdrojů, a to jak pro dospělce, tak i pro larvy (Westerfelt a kol. 2018, Westrich 2018). Pro přítomnost druhů včel hnízdících v ulitách je tedy nezbytným hnízdním zdrojem vysoký podíl prázdných ulit, potravním zdrojem je pak pyl z kvetoucích rostlin. Obojí mohou poskytnout zejména přirozená či přírodě blízká stanoviště (Bogusch a kol. 2020b), která tak mohou sloužit jako ohniska biologické rozmanitosti včel, podobně jako tomu je u malých polních mokřadů ve střední Evropě (Heneberg a kol. 2018). Přítomnost přírodě blízkých stanovišť (a v menší míře i míst s ruderální vegetací) je oboustranně výhodná zejména v okolí sadů, přičemž kvetoucí stromy včelám poskytují další dostupný zdroj potravy, přítomnost těchto opylovačů pak může následně podpořit výnosy plodin pěstovaných v sadech (Bogusch a kol. 2020b).

V rámci studia hnízdní biologie jsme se zaměřili především na druhy vyskytující se v České republice, dále na evropské druhy rodu *Rhodanthidium* a okrajově i na další druhy ulitových včel. Autoři předchozích publikovaných výzkumů se většinou zaměřovali pouze na několik druhů (např. Bellmann 1981, Moreno-Rueda a kol. 2008, Müller 2018), my jsme danou problematiku pojali komplexněji. U našich druhů, u kterých je hnízdní biologie známá, jsme potvrdili předchozí publikované údaje. Například u druhu *O. rufohirta* jsme pozorovali preferenci ulit druhu *X. obvia* a dalších ulit malé velikosti (Heneberg a kol. 2020), což je v souladu s tím, co zjistil Bellmann (1981). Druh *O. aurulenta* dle Müllera a kol. (2018) kolonizuje ulity střední až velké velikosti. Toto tvrzení jsme potvrdili, naše výzkumy však navíc přinesly zjištění, že velké ulity druhu *H. pomatia* byly preferované více než střední ulity *C. vindobonensis*, a to i přesto, že počty ulit těchto dvou druhů byly na zkoumaných lokalitách přibližně stejné (Bogusch a kol. 2019, Heneberg a kol. 2020). Druh *O. spinulosa* byl předchozími publikovanými údaji označen za specialistu na hnízdění v ulitách malých až středních velikostí (Müller 2018), během našich výzkumů ale akceptoval stejně často malé ulity *X. obvia* a velké ulity *H. pomatia* (Bogusch a kol. 2019, Heneberg a kol. 2020).

Zcela jiná situace byla u našeho druhu *R. septemdentatum* a obecně u všech čtyř evropských druhů tohoto rodu, které hnízdí v ulitách. Jedná se o druhy *R. septemdentatum*, *R. sticticum*, *R. siculum* (Spinola, 1838) a *R. infuscatum* (Erichson, 1835) (Kasparek 2019). Jejich hnízdní biologie byla donedávna prozkoumaná jen velmi okrajově a například informace o počtu plodových komůrek jednotlivých druhů byla doposud neznámá (Pasteels 1977, Ortiz-

Sánchez 1990). Pouze druh *R. siculum* byl studován podrobněji, Erbar a Leins (2017) se však zaměřili na popis hnízdního chování, nikoliv na strukturu hnízda samotného. Náš výzkum je tedy první, který přinesl informace o struktuře hnízd těchto čtyř druhů (Hostinská a kol. 2021).

Během našich výzkumů ve Španělsku se nám dále podařilo získat větší množství hnízd druhů *H. fertoni* a *O. ferruginea*. Biologie ulitových včel v jižní Evropě je obecně velmi málo známá. Struktury hnízd obou zmíněných druhů již byly publikovány předešlými autory (Ferton 1905, 1908, Benoist 1931, Grandi 1961, Haeseler 1997, Le Goff 2003, Moreno-Rueda a kol. 2008, Müller a kol. 2018, Müller 2021), naše výsledky tyto známé informace potvrdily, a navíc přinesly nová zjištění. Některá hnízda byla parazitována, například v hnízdech *H. fertoni* jsme nalezli zlatěnku *C. hybrida*, kterou u daného druhu pozoroval i Le Goff (2003). Dále jsme v hnízdech *H. fertoni* objevili kukaččí včelu *D. moesta*, přičemž v tomto případě se jedná o vůbec první záznam parazitace v hnízdech ulitových včel. U druhu *O. ferruginea* jsme zcela nově pozorovali parazitické druhy *S. quinquepunctata* a *A. aethiops* (Bogusch a kol. 2020c). Náš výzkum přispěl k doplnění údajů přinesením nových záznamů o parazitech, pylu, životním cyklu a znacích hnízdního chování. Hnízdní chování, preference na pyl a hostitelsko-parazitické asociace jsou důležitým zdrojem informací pro rekonstrukci fylogeneze u včel čeledi Megachilidae.

V rámci výzkumů stepních druhů jsme sledovali změny rozšíření našich ulitových včel a porovnávali je se změnami rozšíření vybraných druhů rodu *Eucera,* které hnízdí v zemi. Ukázalo se, že druhy hnízdící v prázdných ulitách se v rámci ČR rozšířily, ale druhy hnízdící v zemi z naší fauny naopak mizí (Bogusch a kol. 2020a). Změny v rozšíření druhů souvisí s obecnými změnami krajiny, ke kterým došlo přibližně během posledních 100 let, a jsou spojeny se změnou půdy zemědělstvím a lesnictvím (Jongman 2002, Walz 2008, Skaloš a kol. 2011, Skokanová a kol. 2012). Písčité biotopy a oblasti, které bylo nemožné použít k výsadbě plodin, byly zalesněny (Balej 2012, Lorencová a kol. 2013). Dokonce i mnoho chráněných lokalit bylo napadeno a zarostlo invazními rostlinami, protože úsilí ochrany přírody nebylo dostatečné (Tickle 2000, Oszlányi a kol. 2004). V důsledku toho se zbytky stepních útvarů změnily v křovinaté stráně nebo louky (Lorencová a kol. 2013). Tyto změny na stepích jsou spojeny s úplnou absencí holé půdy, s tvrdým podkladem nevhodným pro hnízdění a nižší diverzitou kvetoucích rostlin. Nedostatek hnízdišť a horší dostupnost kvetoucích rostlin jakožto zdroje potravy pak měly za následek podstatné snížení početnosti nebo vyhynutí populací druhů hnízdících v zemi (Westrich 1996, Kosior a kol. 2007, Přidal & Veselý 2011, Nieto a kol. 2014, Rasmont a kol. 2015). Oproti tomu vysoká produkce křovité vegetace přerůstající zbytky stepí poskytla vhodné podmínky pro mnoho větších druhů plžů (např. *H. pomatia*, *C. vindobonensis*,

*F. fruticum*), které na těchto lokalitách zvýšily své populační hustoty (Peltanová a kol. 2012, Juřičková a kol. 2014). Naopak na ranně sukcesní stádia na antropogenních stanovištích (bývalé vojenské prostory, silniční a železniční náspy, pískovny apod.) se rozšířily menší druhy plžů, jejichž ulity jsou ale stále vhodné pro zahnízdění včel (např. *X. obvia, M. cartusiana*). Jestliže přítomnost a početnost hnízdních příležitostí, jako např. prázdných ulit, je nejvíce limitujícím faktorem pro ulitové včely, pak vzrůst dostupnosti ulit je očekávaná podmínka pro šíření druhů hnízdících v ulitách (Bogusch a kol. 2019). Důležitou otázkou ochrany je, zda můžeme udělat něco pro to, aby byly stepní formace opět vhodné jak pro druhy hnízdící v zemi, tak pro druhy hnízdící v ulitách. V poslední době díky implementaci vhodných ochranných opatření došlo ke zlepšení této situace a je tedy možné, že se některé stepní biotopy, zejména ty s patřičnými managementovými zásahy, brzy stanou vhodnými i pro hnízdění druhů v zemi. Nicméně půjde o dlouhotrvající proces vyžadující pravidelnou implementaci nových managementových postupů (Bogusch a kol. 2020a).

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# 5 PŘÍLOHY

**5.1 Bogusch P., Roháček J., Baňař P., Astapenková A., Kouklík O., Pech P., Janšta P., Heller K., Hlaváčková L., Heneberg P. 2019: The presence of high numbers of empty shells in anthropogenic habitats is insufficient to attract shell adopters among the insects.** ***Insect Conservation and Diversity*** **12: 193–205. https://doi.org/10.1111/icad.12335.**

V prvním roce jsme se zaměřili na studium ulit a jejich využívání různými bezobratlými na stepích a antropogenních stanovištích (v ČR a Maďarsku), a to zejména z hlediska přezimování. Zkoumané lokality byly dvojího typu – step či lesostep a přilehlé postindustriální stanoviště, např. lomy, jíloviště, pískovny, železniční či silniční náspy nebo vinice (celkem 25 párů a dále 7 lokalit, které nebyly spárované).

Sběr ulit probíhal od 28. prosince 2015 do 18. března 2016. Na každé lokalitě jsme sesbírali minimálně 400 prázdných ulit ležících na povrchu země. Spektrum sbíraných ulit plně odráželo spektrum velkých a středních ulit dostupných na dané lokalitě a z hlediska velikosti považovaných za vhodné pro hnízdění žahadlových blanokřídlých. Ulity z jednotlivých lokalit byly následně dopraveny do laboratoře, kde byly roztříděny dle druhů plžů a umístěny do označených boxů. Boxy byly pravidelně kontrolovány a vylíhlí členovci (především hmyz a pavouci) byli odebíráni, fixováni v ethanolu a následně určeni.

Druhově nejpočetnějším se ukázal řád blanokřídlých (Hymenoptera). Dále byly zastoupeny řády polokřídlí (Hemiptera), dvoukřídlí (Diptera) a brouci (Coleoptera). Jedním až několika druhy byly zastoupeny také řády motýli (Lepidoptera), síťokřídlí (Neuroptera), dlouhošíjky (Raphidioptera), škvoři (Dermaptera) a švábi (Blattodea). Z řádu blanokřídlých (Hymenoptera) se v ulitách vyskytovali především zástupci čeledí zlatěnkovití (Chrysididae), čalounicovití (Megachilidae), hrabalkovití (Pompilidae), drvenkovití (Sapygidae) a mravencovití (Formicidae). Z ulitových včel byly zaznamenány druhy *Osmia aurulenta, O. bicolor, O. rufohirta, O. spinulosa,* z jejich parazitů pak kukaččí včela *Stelis odontopyga,* zlatěnky *Chrysura dichroa, C. trimaculata* a drvenka druhu *Sapyga quinquepunctata*.

Tímto výzkumem jsme vůbec poprvé zpracovali ucelený přehled hmyzu a pavouků, kteří využívají prázdné ulity k přezimování ve střední Evropě. Ukázalo se, že hmyz využívající ulity se specializuje na konkrétní druhy plžů pouze vzácně, ulity jsou vybírány výrazně častěji podle jejich typu (velikosti apod.). Dále jsme zjistili, že přítomnost prázdných ulit na antropogenních stanovištích umožňuje přítomnost mnoha druhů hmyzu, které ulity využívají,

ovšem zároveň se ukázalo, že samotný výskyt ulit k rozšíření veškerého ulity využívajícího hmyzu z přírodních na antropogenní stanoviště nestačí. Vyhýbání se antropogenním lokalitám bylo zřetelné zejména u hmyzu, který ulity využívá pouze jako místo k přezimování a který vyžaduje další potravní a hnízdní zdroje – například ploštice (Heteroptera).

Insect Conservation and Diversity

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# The presence of high numbers of empty shells in anthropogenic habitats is insufficient to attract shell adopters among the insects

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**Abstract.** 1. Terrestrial snail shells can be considered partial resources for insects, and as such, the expansion of numerous snail species to anthropogenic habitats makes them increasingly available.

2. Here, we address for the first time a complete profile of insects that use empty terrestrial snail shells during the winter period in Central Europe.

3. The specialisation for shells made by certain snail species was uncommon; however, a number of species showed significant preferences for certain shell types. We found that the presence of empty snail shells in anthropogenic habitats drives the presence of many empty snail shell adopters in these habitats. Nevertheless, the increased availability of snail shells proved to be insufficient for a transition of all the species of snail shell adopters from natural to anthropogenic habitats. The avoidance of anthropogenic habitats among snail shell adopters was particularly distinct in species that use them only as a winter retreat but which require additional feeding and breeding resources, such as the true bugs.

4. The availability of snail shells is thus a pre-requisite of the presence of specialised snail shell adopters but is not necessarily sufficient to establish their presence in the respective habitat.

**Key words.** Aculeate Hymenoptera, breeding resources, expansive species, roadside verges, snail predators, *Temnothorax*, terrestrial Gastropoda.

## Introduction

With the onset of industrialisation, anthropogenic landscapes dominate many regions of the world. The anthropogenic landscapes include numerous brownfields, open-cast mines and quarries, waste dumps and various linear elements, such as roadside verges. The mosaic of above-named habitats has been colonised by a specific assemblage of mollusc species. In Central Europe, these are represented mainly by *Xerolenta obvia*, *Cepaea* spp., and *Helix pomatia* (Alexandrowicz & Alexandrowicz, 2010; Rosin *et al.*, 2017); in the Pannonian region, *Cepaea vindobonensis* and *Zebrina detrita* are common in anthropogenic habitats as well (P. Bogusch & P. Heneberg, pers. obs.). In other regions, *Theba pisana* is among the typical species that preferentially occur in roadside verges (Odendaal *et al.*, 2008). Available evidence suggests passive transport of these species by means of multiple common human activities (Dörge *et al.*, 1999), and these species may benefit from other changes in landscape

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use, such as pasture abandonment (Labaune & Magnin, 2002).

Empty terrestrial snail shells host a diverse but poorly understood community of insects and other arthropods. Among the taxa that are specialised for empty snail shells, the aculeate Hymenoptera are likely the best understood (Müller *et al*., 2018). Immature stages of saprophagic, predatory, and parasitic dipterans develop on the dead or alive snails and later overwinter in the shells. Particularly, the snail-killing flies of the family Sciomyzidae are largely malacophagous, and intense research has been conducted in the past in order to establish them as biological control agents of introduced snails that act as agricultural pests. Besides the dipterans, the empty snail shells may host the predatory larvae of Drilidae (Coleoptera), with *Drilus concolor* and *Drilus flavescens* considered predators of helicid snails. Reports on the presence of other groups of insects in snail shells are nearly absent except of reports of parasites and parasitoids of the above-named insects, such as *Mesoleptus* spp. that parasitise Sciomyzidae (Jussila *et al*., 2010). See ESM2 for an overview of the previously published data.

Here, we provide for the first time a complete profile of insects that use empty terrestrial snail shells during the winter period, with the focus on steppes and allied habitats of Central Europe. We hypothesised that the increased availability of empty snail shells in multiple anthropogenic habitats facilitates the presence of empty snail shell adopters in these habitats as is shown in several anthropogenic habitats for the *Osmia* bees (Heneberg *et al*., 2013, 2017). We tested whether they reach similar diversity and abundance in natural habitats (steppes and forest steppes) and in open anthropogenic habitats as calculated per the number of examined shells. Furthermore, we hypothesised that the occupancy of shells is constrained by snail species and, thus, that this specialisation contributes to the presence of a variety of breeding niches within each sampling site. Based on the obtained evidence, we discuss the limitations of empty snail shells as breeding resources in anthropogenic habitats with regard to the concept of partial habitats (Westrich, 1996).

## Materials and methods

### *Study area and sampling sites*

The study was carried out at sampling sites that were arranged in pairs, each containing one natural steppe or forest-steppe paired with a nearby anthropogenic habitat. The distance between paired sampling sites was 4.8 ± 6.4 km (range 0.1–20.2 km). The distribution of sampling sites was not random; it reflected the distribution of steppic fragments across the study area and our knowledge of sites where empty shells or shell-specialised aculeates were present in the past. The sampling sites mostly involved karstic areas, extinct volcanoes, a substantial number of sampling sites were located in loess deposits and, surprisingly, several sites rich in shells were present in areas composed of silicates – quartzite rock steppes and sand pits.

The study included 25 pairs of sampling sites. In addition, we examined seven sampling sites that did not fulfil the criteria of paired sampling sites due to insufficient numbers of snail shells being present either at the examined site itself or at the proposed paired site. The data obtained at these sites were included in the total counts but not in the paired tests. All study sites were located in Central Europe: 21 sampling sites were in the Pannonian biogeographic region (SE Czech Republic and N Hungary), 34 sampling sites were in the Continental biogeographic region (Czech Republic), and two sampling sites were at the edge of the Alpine (West Carpathian) biogeographic region (E Czech Republic; Fig. 1). Detailed list of sampling sites and their coordinates are provided in Table S1. All examined natural habitats consisted of steppes or forest steppes. The examined anthropogenic habitats consisted of stone quarries ($n = 13$), clay pits ($n = 4$), sand pits ($n = 4$), lignite spoil heaps ($n = 3$), railway verges ($n = 1$), roadside verges ($n = 1$), and vineyards ($n = 1$). The sampling sites were chosen to represent the whole spectrum of sites with abundant presence of empty snail shells known to us throughout the study area.

### *Sampling*

Shells were collected from December 28, 2015 to March 18, 2016. At each examined paired site, we collected at least 400 empty terrestrial snail shells (mean 534; range 428–704) that were visible on the surface. The spectrum of collected shells (Fig. 1b) fully reflected the spectrum of large and medium-sized shells available at the examined sites and considered suitable for the nesting of aculeate hymenopterans due to size limitations. We non-selectively collected empty large- and medium-sized shells of any species present on the soil surface. However, we omitted shells of juvenile *Xerolenta obvia* and similar or smaller shells of other species that are clearly unsuitable for the nesting of aculeate hymenopterans due to size limitations. The sampling could potentially be biased against the species that use only shells present deep in the leaf litter, under stones or in soil burrows. Insects were reared exposed to a daylight cycle, at temperatures between 15–23 °C for 3–4 months. The reared insects were fixed in ethanol. The total number of shells sampled reached 27 650, out of which 13 937 (50.4%) were obtained from natural steppic habitats, and 13 713 (49.6%) were collected from anthropogenic habitats.

### *DNA barcoding*

For the species confirmation of *Bradysia nigrispina* and *Philosepedon humeralis*, we employed DNA barcoding.

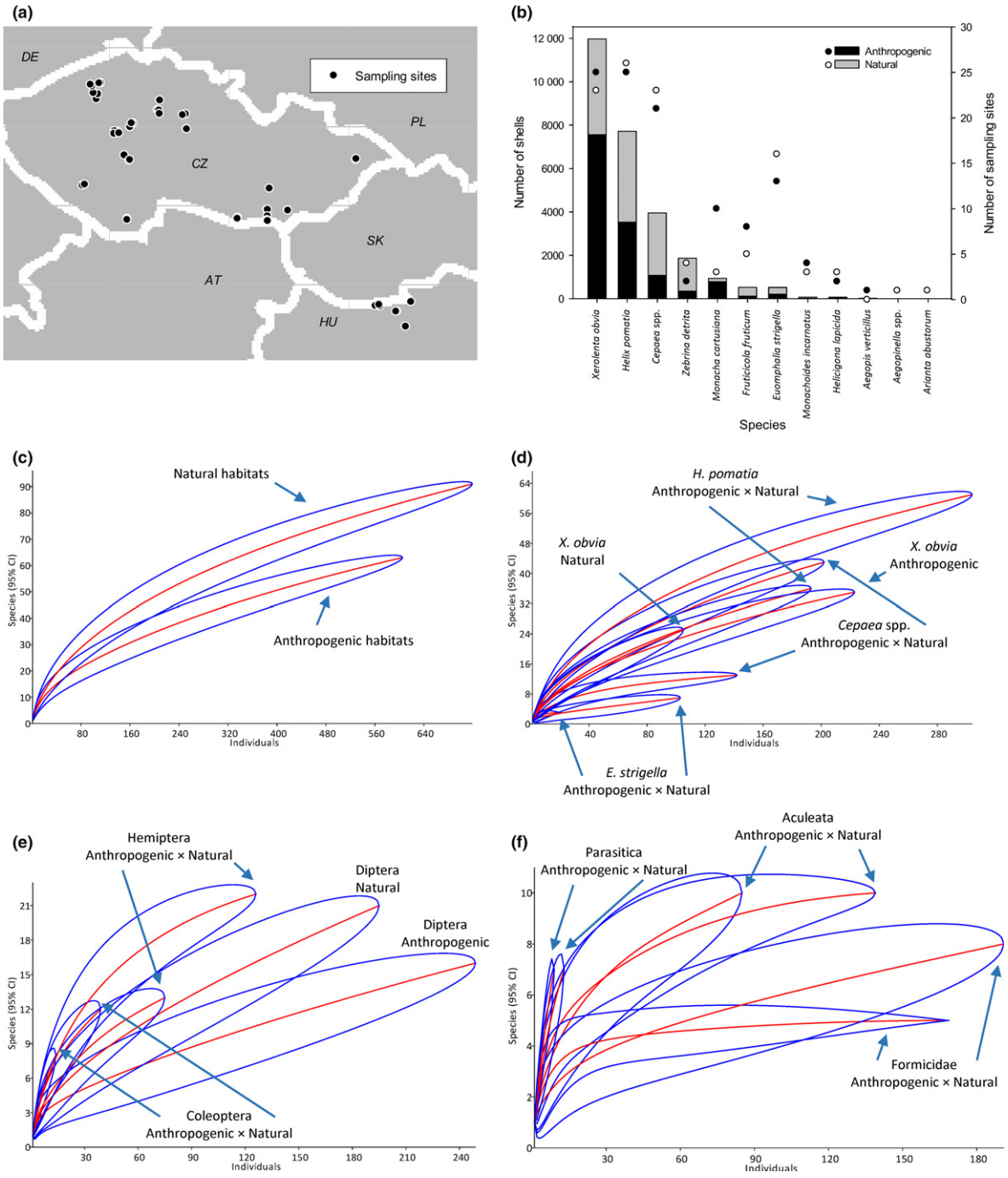



**Fig. 1.** Location of study sites, the numbers of materials examined and the rarefaction curves of reared insects. (a) Location of study sites in Central Europe. Black dots represent pairs of sampling sites and the additional sites, for which the paired sites were not available. (b) Number of shells collected in the course of the present study (stacked columns) and numbers of sampling sites at which the shells of the respective species were available (dots). Data are presented separately for anthropogenic (black) and natural (white dots, grey columns) sampling sites. The frequencies of collected shell species correspond to the frequencies of shells that were visible at the examined sites. (c) Rarefaction curve of insects reared from shells of terrestrial snails collected in natural and anthropogenic habitats. (d) Rarefaction curve of insects reared from shells of *Cepaea* spp., *Helix pomatia*, and *Xerolenta obvia*, with the data from natural and anthropogenic habitats compared. (e) Rarefaction curve of Diptera, Coleoptera, and Hemiptera reared from shells of terrestrial shells, with the data from natural and anthropogenic habitats compared. (f) Rarefaction curve of Hymenoptera: Aculeata except Formicidae, Formicidae, and Parasitica reared from shells of terrestrial shells, with the data from natural and anthropogenic habitats compared. [Colour figure can be viewed at wileyonlinelibrary.com].

The DNA was isolated and the CO1 locus was amplified and sequenced as described by Heller and Rulik (2016). The barcoded ethanol-fixed voucher specimens are available from the Alexander Koenig Museum in Bonn. All sequences were deposited in BOLD (https://doi.org/10.5883/ds-czdipsna) and in the NCBI Nucleotide database (under accession numbers MH917737-MH917740).

### *Statistical analyses*

All insects obtained in the course of the rearing experiments were included in the analyses. To estimate their species richness, we calculated the Chao-1 estimator, corrected for unseen species and plotted the rarefaction curves. To compare species richness of the analysed datasets, we calculated the Sørensen, Morisita-Horn and the combined Chao's Sørensen raw (uncorrected for unseen species) abundance-based similarity (Chao *et al.*, 2005, equation 6) indices. We also calculated total numbers of species and individuals found, and the basic diversity indices, including the dominance ($D$ = 1 – Simpson index), equitability, Fisher's alpha, and Berger-Parker dominance indices. Pearson and Spearman correlation coefficients and their significance were calculated when indicated. $\chi^2$ test with Yates correction was used to analyse the differences in abundance. The conservation value of analysed species was assessed according to the most recent version of the national Red List (Hejda *et al.*, 2017). Neuroptera, Raphidioptera, and Diptera are absent in the most recent Red List edition; thus, we used the previous Red List version (Farkač *et al.*, 2005) to evaluate the conservation value of these taxa. The species included in the Czech Red List were termed as 'threatened' throughout the text and include species known as vulnerable (VU) or near threatened (NT). All the calculations were performed in SigmaPlot 12.0 (Systat Software, San Jose, CA, USA). EstimateS 9.1.0 (University of Connecticut, Storrs, CT, USA) and PAST 2.14 (University of Oslo, Oslo, Norway). Data are shown as mean ± SD unless stated otherwise.

## Results

### *Global view on the empty shell universe*

In total, 1324 insects were reared from 27 650 collected shells. The shells hosted 117 morphospecies of insects of nine orders (Table S2). The examined assemblages were characteristic by large number of species that we found only in low numbers. Thus, the Chao-1 species richness estimator (corrected for unseen species in the samples) indicated a species richness of 192.5 ± 28.9 species, suggesting that there are another 75 species that are waiting to be collected from snail shells in the study region. There was no eudominant species within the analysed dataset as indicated by the low dominance value ($D$ = 0.079; Berger-Parker = 0.162); instead, a group of several specialised species dominated the examined assemblages. Correspondingly, the overall alpha diversity of the study assemblages was high, with Fisher's alpha reaching 30.96. However, the equitability reached only 0.67 suggesting that the entropy (the evenness with which individuals are divided among the taxa present) of the study assemblages was low. Collectively, these indices show that although the study assemblages were diverse, they were dominated by a group of several species (Fig. 2). The abundantly present insects consisted of dipterans of the Psychodidae, Sciaridae, Sciomyzidae, and Anthomyiidae families, several megachilid bees and cuckoo wasps, various ant species and true bugs of the family Rhyparochromidae. The beetles were dominated by *Oulema melanopus*. Species from other orders of insect were represented by just a few individuals (Fig. 3, Table S2). Below, we will focus on possible drivers of low entropy among the study assemblages and on the role of anthropised systems in their distribution.

### *Is the availability of shells in anthropogenic habitats sufficient to attract their adopters?*

From the reared individuals, 605 individuals emerged from the shells collected at anthropogenic sites (4.4 individuals per 100 shells collected), and a slightly higher amount of 719 individuals emerged from the shells collected at natural sites (5.2 individuals per 100 shells collected). Thus, the abundance of insects in empty shells at natural and anthropogenic sites differed from the expected equal abundance per shell ($\chi^2$ test with Yates correction $\chi^2$ = 3.938, d.f. = 1, $P$ = 0.047).

The Chao-1 species richness estimator (corrected for unseen species in the samples) indicated a species richness of 111.5 ± 24.2 species in anthropogenic habitats and 148.9 ± 26.1 species in natural habitats, which was also supported by the rarefaction of the two datasets (Fig. 1c). The communities differed significantly in the number of species found (63 vs. 90 species in anthropogenic and natural habitats, respectively, bootstrapping $P$ = 0.001). The dominance was slightly but significantly higher in anthropogenic habitats ($D$ = 0.110 vs. 0.081; bootstrapping $P$ = 0.001). Correspondingly, the overall alpha diversity of the study assemblages was lower in anthropogenic habitats, with Fisher's alpha reaching 17.69 and 27.12 (bootstrapping $P$ = 0.001) respectively. Interestingly, the equitability differed only marginally (0.67 vs. 0.72; bootstrapping $P$ = 0.004). The Chao estimated number of shared species was 58 (35 directly observed), the Sørensen similarity index that reflects the presence/absence data was just 0.463, but the Morisita-Horn index was only slightly higher, at 0.677. The combined Chao's Sørensen raw (uncorrected for unseen species) abundance-based similarity reached 0.876 (estimated reached 0.946) suggesting that the differences in insect assemblages adopting snail shells at the two habitat types do not stem from the absence of species in one of

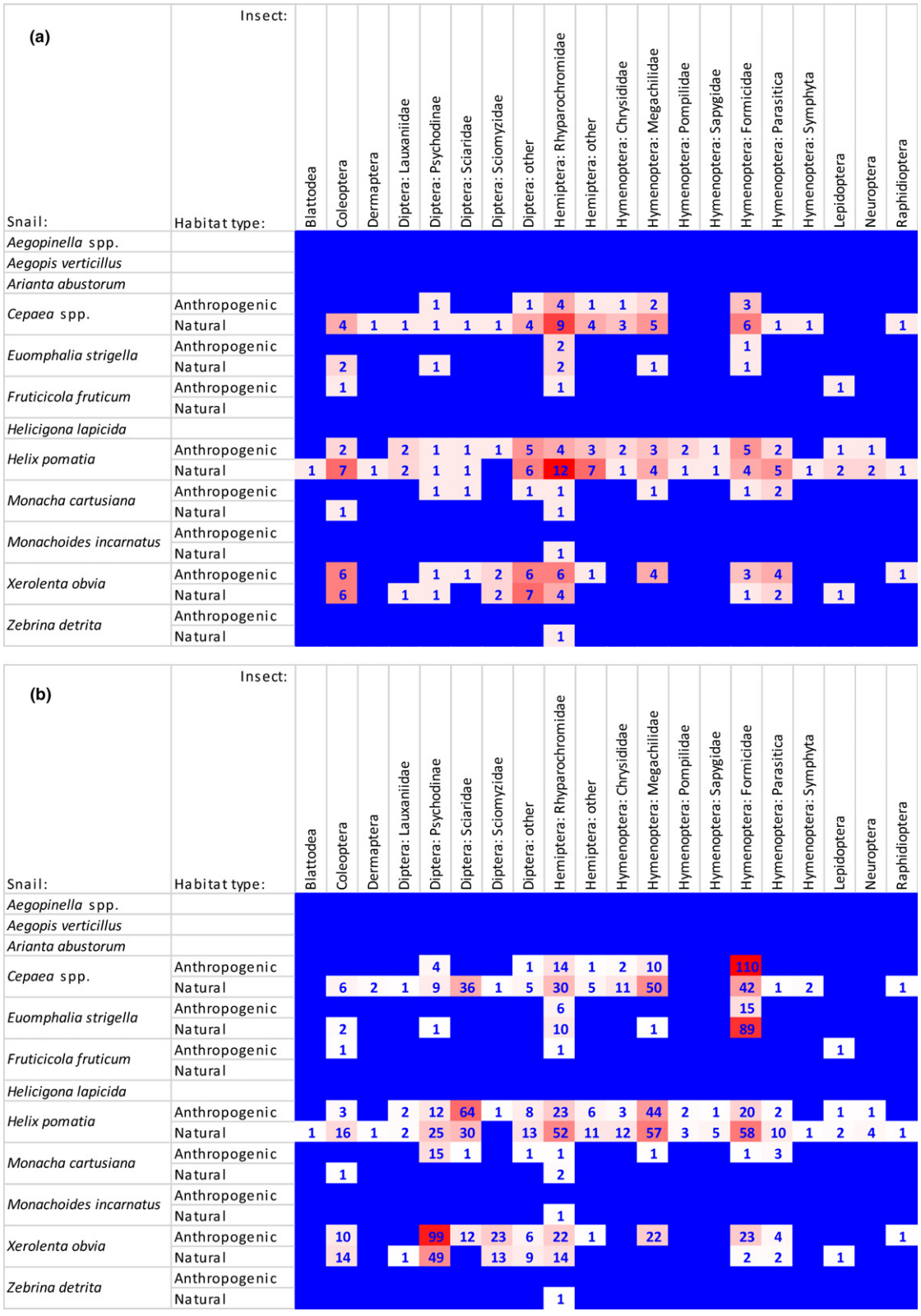
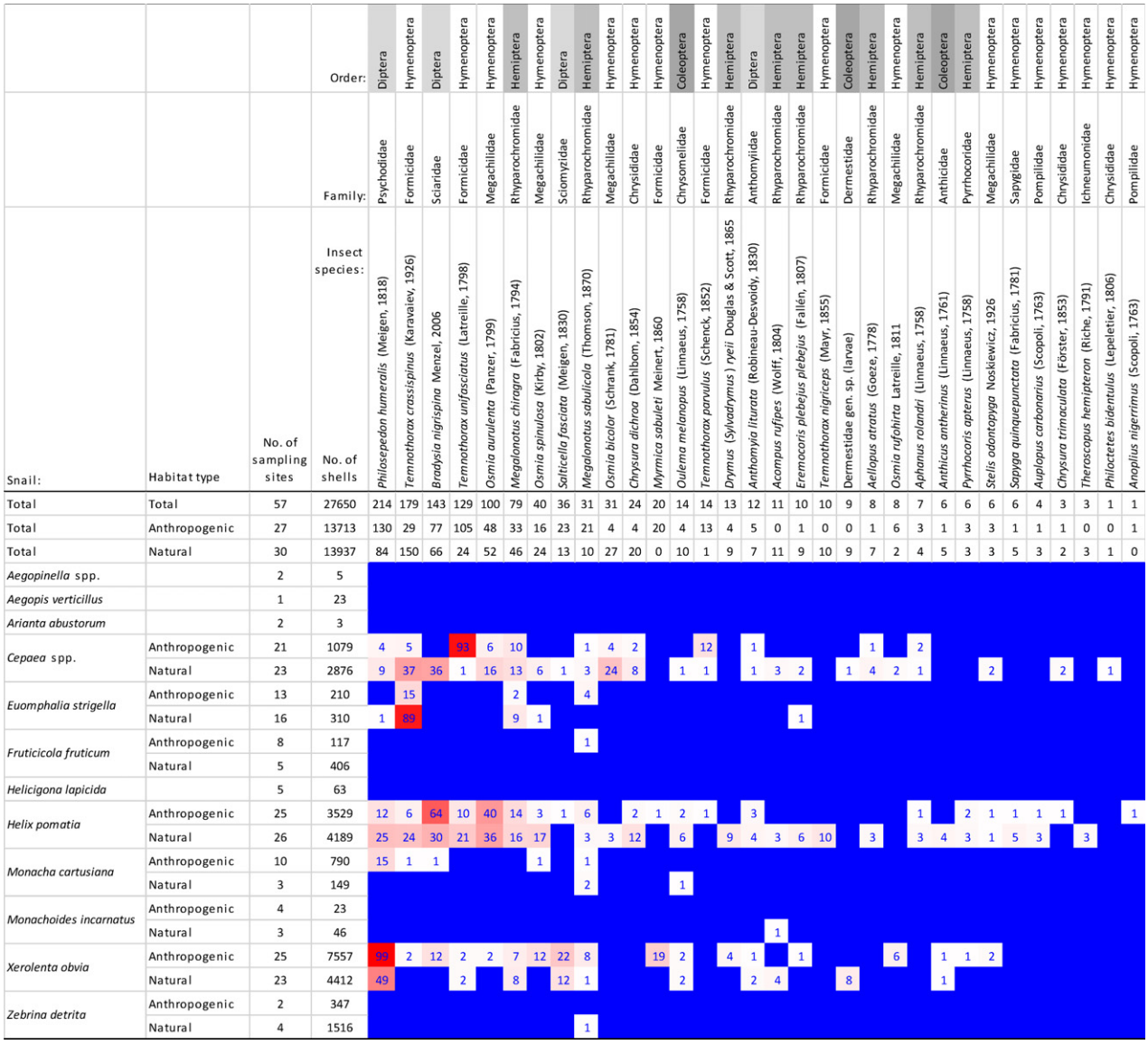



**Fig. 2.** Heat maps of the numbers of species (a) and individuals (b) of insect orders and selected lower taxonomical units that were reared from collected shells of terrestrial snails. The snail species and habitat types are indicated. [Colour figure can be viewed at wileyonlinelibrary.com].



| Snail: | Habitat type | No. of sampling sites | No. of shells | Order: | Diptera | Hymenoptera | Diptera | Hymenoptera | Hymenoptera | Hemiptera | Hymenoptera | Diptera | Hemiptera | Hymenoptera | Hymenoptera | Hymenoptera | Coleoptera | Hymenoptera | Hemiptera | Diptera | Hemiptera | Hemiptera | Hymenoptera | Coleoptera | Hemiptera | Hymenoptera | Hemiptera | Coleoptera | Hemiptera | Hymenoptera | Hymenoptera | Hymenoptera | Hymenoptera | Hymenoptera | Hymenoptera | Hymenoptera |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Family: | Psychodidae | Formicidae | Sciaridae | Formicidae | Megachilidae | Rhyparochromidae | Megachilidae | Sciomyzidae | Rhyparochromidae | Megachilidae | Chrysididae | Formicidae | Chrysomelidae | Formicidae | Rhyparochromidae | Anthomyiidae | Rhyparochromidae | Rhyparochromidae | Formicidae | Dermestidae | Rhyparochromidae | Megachilidae | Rhyparochromidae | Anthicidae | Pyrrhocoridae | Megachilidae | Sapygidae | Pompilidae | Chrysididae | Ichneumonidae | Chrysididae | Pompilidae |
| | | | | Insect species: | *Philosepedon humeralis* (Meigen, 1818) | *Temnothorax crassispinus* (Karavaiev, 1926) | *Bradysia nigrispina* Menzel, 2006 | *Temnothorax unifasciatus* (Latreille, 1798) | *Osmia aurulenta* (Panzer, 1799) | *Megalonotus chiragra* (Fabricius, 1794) | *Osmia spinulosa* (Kirby, 1802) | *Saltiella fasciata* (Meigen, 1830) | *Megalonotus sabulicola* (Thomson, 1870) | *Osmia bicolor* (Schrank, 1781) | *Chrysura dichroa* (Dahlbom, 1854) | *Myrmica sabuleti* Meinert, 1860 | *Oulema melanopus* (Linnaeus, 1758) | *Temnothorax parvulus* (Schenck, 1852) | *Drymus* (*Sylvadrymus*) *pyeii* Douglas & Scott, 1865 | *Anthomyia liturata* (Robineau-Desvoidy, 1830) | *Acompus rufipes* (Wolff, 1804) | *Eremocoris plebejus plebejus* (Fallén, 1807) | *Temnothorax nigriceps* (Mayr, 1855) | Dermestidae gen. sp. (larvae) | *Aellopus atratus* (Goeze, 1778) | *Osmia rufohirta* Latreille, 1811 | *Aphanus rolandri* (Linnaeus, 1758) | *Anthicus antherinus* (Linnaeus, 1761) | *Pyrrhocoris apterus* (Linnaeus, 1758) | *Stelis odontopyga* Noskiewicz, 1926 | *Sapyga quinquepunctata* (Fabricius, 1781) | *Auplopus carbonarius* (Scopoli, 1763) | *Chrysura trimaculata* (Förster, 1853) | *Therscopus hemipteron* (Riche, 1791) | *Philoctetes bidentulus* (Lepeletier, 1806) | *Anoplius nigerrimus* (Scopoli, 1763) |
| Total | Total | 57 | 27650 | | 214 | 179 | 143 | 129 | 100 | 79 | 40 | 36 | 31 | 31 | 24 | 20 | 14 | 14 | 13 | 12 | 11 | 10 | 10 | 9 | 8 | 8 | 7 | 6 | 6 | 6 | 6 | 4 | 3 | 3 | 1 | 1 |
| Total | Anthropogenic | 27 | 13713 | | 130 | 29 | 77 | 105 | 48 | 33 | 16 | 23 | 21 | 4 | 4 | 20 | 4 | 13 | 4 | 5 | 0 | 1 | 0 | 0 | 1 | 6 | 3 | 1 | 3 | 3 | 1 | 1 | 1 | 0 | 0 | 1 |
| Total | Natural | 30 | 13937 | | 84 | 150 | 66 | 24 | 52 | 46 | 24 | 13 | 10 | 27 | 20 | 0 | 10 | 1 | 9 | 7 | 11 | 9 | 10 | 9 | 7 | 2 | 4 | 5 | 3 | 3 | 5 | 3 | 2 | 3 | 1 | 0 |
| *Aegopinella* spp. | | 2 | 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Aegopis verticillus* | | 1 | 23 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Arianta abustorum* | | 2 | 3 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Cepaea* spp. | Anthropogenic | 21 | 1079 | | 4 | 5 | | 93 | 6 | 10 | | | 1 | 4 | 2 | | | 12 | | 1 | | | | | 1 | | 2 | | | | | | | | | |
| | Natural | 23 | 2876 | | 9 | 37 | 36 | 1 | 16 | 13 | 6 | 1 | 3 | 24 | 8 | | 1 | 1 | | 1 | 3 | 2 | | 1 | 4 | 2 | 1 | | | 2 | | | 2 | | 1 | |
| *Euomphalia strigella* | Anthropogenic | 13 | 210 | | | 15 | | | | 2 | | | 4 | | | | | | | | | | | | | | | | | | | | | | | |
| | Natural | 16 | 310 | | 1 | 89 | | | | 9 | 1 | | | | | | | | | | | 1 | | | | | | | | | | | | | | |
| *Fruticicola fruticum* | Anthropogenic | 8 | 117 | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Natural | 5 | 406 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Helicigona lapicida* | | 5 | 63 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Helix pomatia* | Anthropogenic | 25 | 3529 | | 12 | 6 | 64 | 10 | 40 | 14 | 3 | 1 | 6 | | 2 | 1 | 2 | 1 | | 3 | | | | | | | 1 | | 2 | 1 | 1 | 1 | 1 | | 1 |
| | Natural | 26 | 4189 | | 25 | 24 | 30 | 21 | 36 | 16 | 17 | | 3 | 3 | 12 | | 6 | | 9 | 4 | 3 | 6 | 10 | | 3 | | 3 | 4 | 3 | 1 | 5 | 3 | | 3 | | |
| *Monacha cartusiana* | Anthropogenic | 10 | 790 | | 15 | 1 | 1 | | | | 1 | | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Natural | 3 | 149 | | | | | | | | | | 2 | | | | 1 | | | | | | | | | | | | | | | | | | | |
| *Monachoides incarnatus* | Anthropogenic | 4 | 23 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Natural | 3 | 46 | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | |
| *Xerolenta obvia* | Anthropogenic | 25 | 7557 | | 99 | 2 | 12 | 2 | 2 | 7 | 12 | 22 | 8 | | | 19 | 2 | | 4 | 1 | | 1 | | | | 6 | | 1 | 1 | 2 | | | | | | |
| | Natural | 23 | 4412 | | 49 | | | 2 | | 8 | | 12 | 1 | | | | 2 | | | 2 | 4 | | | 8 | | | | 1 | | | | | | | | |
| *Zebrina detrita* | Anthropogenic | 2 | 347 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Natural | 4 | 1516 | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | |

**Fig. 3.** Heat map of the numbers of individuals of the dominant and selected other insect species. The snail species and habitat types are indicated. [Colour figure can be viewed at wileyonlinelibrary.com].

the datasets but rather from differences in their abundance. The lower complexity of insect assemblages in shells at anthropogenic sites was valid for all major orders that occurred in the shells, namely Diptera, Hemiptera, and Coleoptera (Fig. 1e), and for aculeate Hymenoptera (Fig. 1f).

Surprisingly, there was no dominant species occurring at anthropogenic sites only. Of species that were reared in eight or more individuals, only five species were at least twice more common in anthropogenic than natural habitats. These included the ants *Myrmica sabuleti* (20 individuals in only two shells in anthropogenic habitats/0 individuals in natural habitats), *Temnothorax parvulus* (13/1), *Temnothorax unifasciatus* (105/24), the megachilid bee *Osmia rufohirta* (6/2), and a true bug *Megalonotus sabulicola* (21/10; Table S2).

*Does the shell species play a role?*

Only a part of differences in abundance and species richness can be attributed to differences in the numbers of collected shell species. Instead, the three most abundantly occurring shells were occupied by more insect species and more insect individuals per shell than shells from any other species. The highest number of species (77 species) was present in shells of the largest examined species, *Helix pomatia*. They hosted 6.4 insect individuals per 100 examined shells and the associated assemblage displayed the

lowest dominance ($D = 0.08$), the highest diversity (Fisher's $\alpha = 25.50$), and entropy (equitability = 0.74). The second highest number of species (49 species) was retrieved from *Xerolenta obvia*. Despite the insect assemblage that was associated with this small species was species-rich, fewer insects were reared from shells of *X. obvia* compared to other larger snail shells, with only 2.7 individuals reared per 100 examined shells. The insect assemblage associated with *X. obvia* shells displayed higher dominance ($D = 0.23$), and correspondingly lower diversity (Fisher's $\alpha = 15.96$) and entropy (equitability = 0.62) compared to that in *H. pomatia* shells. The third abundant type of shells, *Cepaea* spp., hosted 43 insect species. These shells were associated with a higher insect abundance (8.7 individuals per 100 examined shells) when compared to the two previously mentioned shell types. The insect assemblages in *Cepaea* spp. shells exhibited low dominance ($D = 0.12$) and high entropy (equitability = 0.71), but, in contrast to *H. pomatia* shells, they were less diverse (Fisher's $\alpha = 12.97$). Regarding shells of other snail species, the species richness and particularly the abundance of insects were exponentially lower in shells of *Zebrina detrita*, *Fruticicola fruticum*, *Helicigona lapicida*, and *Monachoides incarnatus* (Table S3). In contrast, the shells of *Euomphalia strigella* and *Monacha cartusiana* were occupied by similarly diverse insect assemblages as shells of the three dominant species. The number of collected shells of *Aegopinella* spp., *Aegopis verticillus*, and *Arianta arbustorum* was insufficient to provide any conclusions; however, based on our observations in the spring, the shells of *Aegopis verticillus* are frequently occupied by *Osmia bicolor* when available (Heneberg, unpubl.). The rarefaction revealed that the insect assemblages are more complex in natural than anthropogenic habitats when considering any of the three dominant shell types (Fig. 1d).

The insect assemblages in shells of *Cepaea* spp. were most similar to those in shells of *H. pomatia* (combined Chao's Sørensen raw (uncorrected for unseen species) abundance-based similarity 0.864) and *X. obvia* (0.785). Sharply contrasting were the similarities of insect assemblages in shells of *Cepaea* spp. with those in shells of *M. cartusiana* (0.440) and *E. strigella* (0.430) and further with the remaining species that displayed very limited similarity (<0.040) due to the very restricted spectrum of insects found in their shells. Extensive overlaps among the examined assemblages were corroborated by high Sørensen index values. Nevertheless, high overlap with *Cepaea* spp. was suggested only for *H. pomatia*, but not *E. strigella* and *X. obvia* by means of the Morisita-Horn index, which reached 0.630, 0.262, and 0.173 for the three above-named species respectively. The difference between the Morisita-Horn index and the other two indices can be attributed to the fact that Morisita-Horn index reaches high values only when two assumptions are fulfilled; there must be overlap in terms of the number of shared species and the individual shared species must occur in the similar proportions in both samples.

Next, we examined overlaps among assemblages that were associated with shells of other snail species. We found high total relative abundances of individuals belonging to the shared species when comparing insects from shells of *E. strigella* with those from *M. cartusiana* (Chao's Sørensen raw (uncorrected for unseen species) abundance-based similarity 0.823) and when comparing the insect assemblage in *X. obvia* with those in *H. pomatia* (0.808), *E. strigella* (0.823), and *M. cartusiana* (0.713; Table S4). However, the individual shared species did not occur in the similar proportions in both samples except for the comparison of insect assemblages in *X. obvia* with *M. cartusiana*. This resulted in high Morisita-Horn index (0.912) for the comparison of insect assemblages from shells of *X. obvia* with *M. cartusiana* while we found very low Morisita-Horn index values for the comparisons of the *E. strigella* dataset with either *M. cartusiana* (0.076) or *X. obvia* (0.029). All other insect assemblages displayed low similarity as revealed by all of the three indices. The only exception was the similarity between insects in shells of *E. strigella* and *F. fruticum*, which was characteristic by intermediate similarity in the species presence/absence data (Sørensen 0.363) but very low abundance-based similarity as revealed by the combined Chao's Sørensen raw (uncorrected for unseen species) abundance-based similarity (0.076) and Morisita-Horn (0.025) indices (Table S4).

### *Correlations of the species composition of anthropogenic habitats with nearby natural habitats*

As the insect assemblages at anthropogenic sites exhibited generally less species than their natural counterparts, we aimed to elucidate whether the insect species that occupy the anthropogenic habitats were distributed independently on the presence of nearby habitat patches or whether their abundance was increased at sites where the proximal natural habitat patches were species-rich. We hypothesised that the spillover from natural to proximal anthropogenic sites is the highest at sites with the highest species richness and abundance. For each pair of sampling sites, we thus calculated the proportion of species that were present at both sampling sites within the pair and compared it to the number of species that were present only at one of the paired sites (Fig. 4a). The trend towards the higher number of shared species at sites with higher species richness was not significant (Pearson $r = 0.316$, $P > 0.05$; Spearman $r = 0.175$, $P > 0.05$, $n = 24$). We also plotted the mean abundance of species that were present at both sampling sites within the pair against the number of species that were present only at one of the paired sites (Fig. 4b). These two variables were negatively correlated suggesting that the less abundant species were, surprisingly, more likely to occur at both sites within the examined pairs (Pearson $r = -0.474$, $P < 0.05$; Spearman $r = -0.494$, $P < 0.05$, $n = 18$). The mean abundance was unrelated to the number of species per sample for both the paired sites-specific groups of

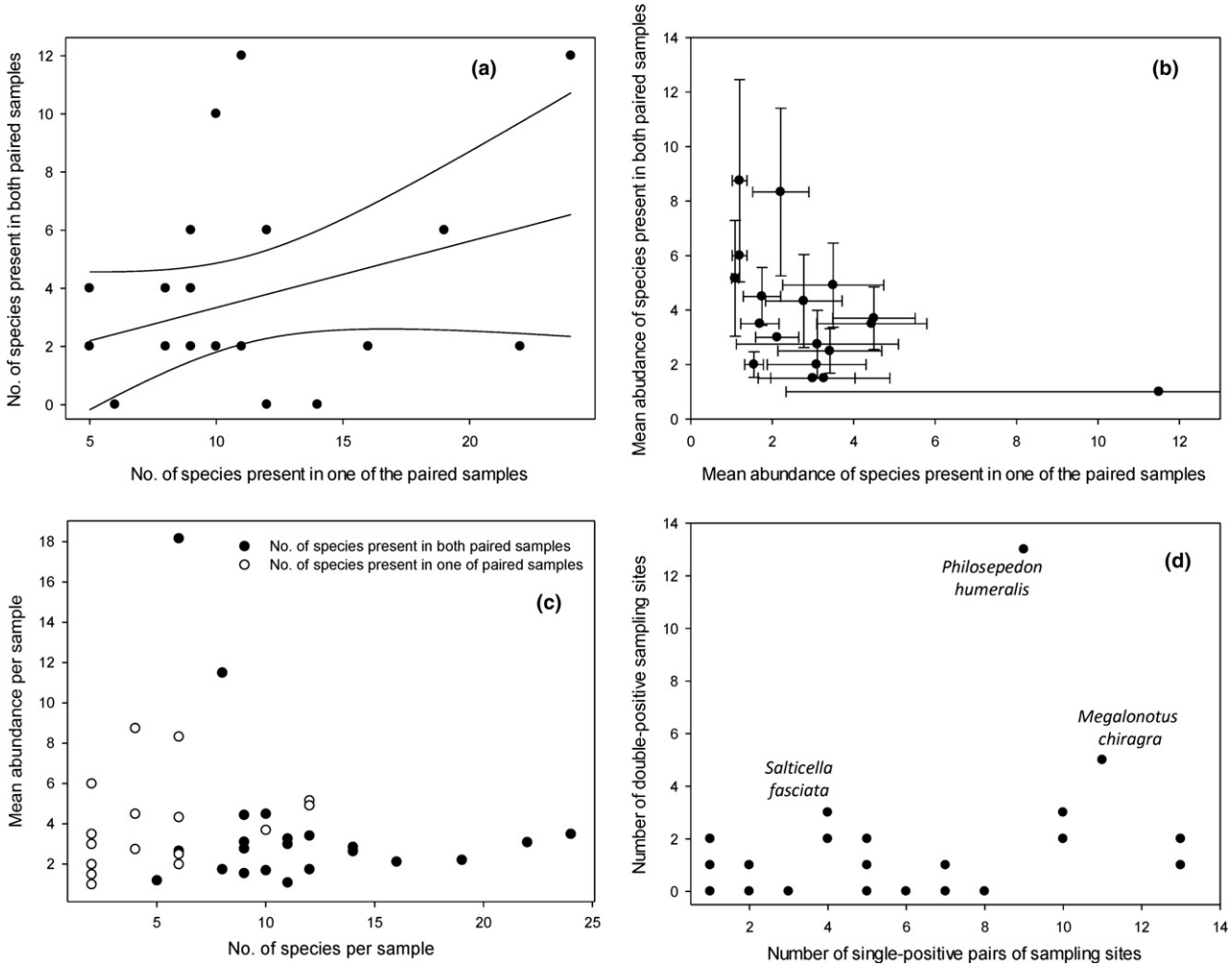



**Fig. 4.** The evaluation of the importance of the species composition of natural habitats that are proximal to anthropogenic habitats. (a) Comparison of the proportion of insect species that were present at both sampling sites within the pair and the number of species present only at one of the paired sites. (b) Mean abundance of insect species that were present at both sampling sites within the pair against the number of species that were present only at one of the paired sites. (c) Mean abundance of insect species plotted against the number of species per sample. Both the paired sites-specific groups of species only present at one of the paired sites and groups of species present at both paired sites are shown. (d) Observed numbers of double-positive paired sites plotted against the numbers of single-positive sampling sites.

species only present at one of the paired sites and the groups of species present at both paired sites (Fig. 4c). From the species-specific point of view, if the random distribution of species was applicable, the expected frequency of double-positive sites would be a power function of their frequency at natural sites. If the proximity of occupied sites played a role, the number of double-positive paired sampling sites would be higher. In contrast, if specific habitat characteristics, which were not shared within the pairs of sampling sites, played a role, the similarity would be lower than expected by the random function. The correlations of the species-specific numbers of single- and double-positive pairs of sampling sites were significant (Pearson $r = 0.508$, $P < 0.001$; Spearman $r = 0.355$, $P < 0.001$, $n = 113$). Although, only a few frequently found species exhibited a higher than random tendency to occur at both paired sites. These included *Salticella fasciata* and *Megalonotus chiragra* (Fig. 4d). It remains to be investigated whether some of the species with limited number of sampling sites belong to this category too – these include, for example, *Stelis odontopyga*, and *Emblethis verbasci*. Combined, these data suggest that the spillover from natural to proximal anthropogenic sites applied only to a part of the species found, whereas the distribution of the majority of species does not correlate with the proximity of nearby natural sites that would host the respective species.

### *Threatened species and new records*

We identified seven threatened species and one species that was new for the Czech Republic. The threatened

species consisted of true bugs (VU: *Megalonotus praetextatus*; NT: *Acompus rufipes*, *Aphanus rolandri*, *Peritrechus nubilus*, and *Pyrrhocoris marginatus*), a bombyliidae diptera (VU: *Spogostylum aethiops*), and a megachilid bee (NT: *Stelis odontopyga*). The threatened true bugs, except *A. rolandri*, were strictly limited to natural sites. The xerothermic bombyliidae *S. aethiops* was restricted to natural sites. The megachilid *S. odontopyga* occurred at natural sites, at lignite spoil heaps, and in the railroad dyke that was adjacent to a steppe. Most of the threatened species, except *M. praetextatus* and *P. marginatus*, were present at multiple sampling sites and in multiple snail species.

The species that we identified as new for the Czech Republic was the sciomyzidae snail-killing fly *S. fasciata*. It was the eighth most abundant species within the examined dataset (Fig. 3). It appears to be specialised for *X. obvia* (34 records); however, we found it in shells of *H. pomatia* (1 record) and *Cepaea* sp. (1 record) as well. It was present both at the natural and anthropogenic sites, mostly at both localities within each pair of sampling sites that was occupied by this fly (Fig. 4d). We recorded it from the Hungarian sampling sites (i.e., from the previously reported distribution area), where it occupied both localities within each *S. fasciata*-positive pair of sampling sites. We newly report it from six sampling sites[1] in South Moravia, the Czech Republic, where it occupied two pairs of sampling sites and two additional anthropogenic sites. The numbers of *S. fasciata* reared from Czech anthropogenic sampling sites were higher than those from the Czech natural sites (Table S2).

*Dominant species*

The examined assemblage was dominated by quite long list of true bugs, four species of flies, and several aculeate hymenopterans. The dominant species of true bugs consisted of a common dry grassland specialists *Megalonotus chiragra*, *M. sabulicola*, and *Aphanus rolandri*, the specialist for wet and shady areas *Drymus* (*Sylvadrymus*) *ryeii*, ubiquitous *Valeriana officinalis* specialist *Acompus rufipes*, species of open forests at sandy or limestone bedrock *Eremocoris plebejus plebejus*, and the common specialist for lime trees and mallows *Pyrrhocoris apterus*. Among the frequently found true bugs also was the expanding species of dry habitats that is often hosted by *Echium vulgare*, *Aellopus atratus*. The majority of records of all dominant true bug species, with the exception of the two *Megalonotus* spp. but including seven of eight records of *A. atratus*, originated from natural habitats only (Fig. 3).

The dominant species of flies were represented by the poorly known species *Bradysia nigrispina*, the viviparous fly that deposits larvae onto the dead snails *P. humeralis*, the above-discussed snail-killing fly *S. fasciata*, and the ubiquitous European species *Anthomyia liturata*. *Philosepedon humeralis* and *S. fasciata* were characteristic particularly for *X. obvia*, whereas the *B. nigrispina* used preferentially *Cepaea* spp. and *H. pomatia*. We confidently identified the abundant species of Sciaridae as *B. nigrispina*, because its holotype was collected in the Czech Republic (Menzel *et al*., 2006) and it is already known from other countries in Central Europe; we also newly provide DNA barcodes of three individuals of this species. However, the taxonomy of this group of species with long antenna and white body hairs, which also includes the cave-dwelling *B. dalmatina*, the alpine *B. pseudodalmatina* and *B. lucida* from Turkmenistan, needs revision in order to clearly separate the species or to discover possible synonymies. *Philosepedon humeralis* was identified by a combined approach involving detailed morphological and molecular analysis. The DNA barcoding confirmed that the sequence of the study species was identical with those of various BINs (BOLD:ABA0882 and BOLD:ACX1419; GBOL, 2018) belonging to *P. humeralis* of European origin. This dipteran species is a specialised inhabitant of dead snail shells (Beaver, 1977). All the dominant dipteran species occurred both at natural and anthropogenic sites although their abundance was lower in the latter habitat type (Table S2).

When considering the aculeate hymenopterans, we need to distinguish between the bees and wasps, which used the shells for nesting, and ants, for which the types of the use of empty shells are more variable. The nesting bees and wasps were dominated by *Osmia aurulenta* that was dominant particularly in *H. pomatia* and occupied frequently *Cepaea* spp., *Osmia spinulosa*, which occupied all the three dominant types of shells, and *Osmia bicolor*, which turned to be a *Cepaea* spp. specialist. While the first two species were present equally in natural and anthropogenic habitats, *O. bicolor* was common only in the natural habitats. The most abundant parasite of their nests was represented by *Chrysura dichroa* that was also abundant particularly in natural habitats and occupied the *Cepaea* spp. and *H. pomatia* shells.

Regarding the ants, most records consisted of workers only, but we also repeatedly recorded nests of ants nesting in small colonies that are characteristic for closed microspaces, such as the *Temnothorax* spp. (Seifert, 2017). The apterous queens were recorded as follows: *Myrmica ruginodis* (three apterous queens), *Temnothorax crassispinus* (four apt. queens), *Temnothorax nigriceps* (one apt. queen), *Temnothorax parvulus* (10 apt. queens), and *Temnothorax unifasciatus* (two apt. queens). In addition to the species recorded in the systematically collected shells, we recently collected also a nest of *Tapinoma erraticum* in summer 2016 a shell collected in Sümeg, Hungary (P. Heneberg & P. Pech, unpubl.).

[1]*Salticella fasciata*, the first Czech records: Čejč, HO – disused claypit, 48.94N, 16.98E, 29-Jan-2016, 7M & 5F in *X. obvia*; Čejč, HO – steppe PP Špidláky, 48.94N, 16.98E, 29-Jan-2016, 1M in *Cepaea* sp., 1F in *X. obvia*; Mikulov, BV – forest steppe PR Svatý kopeček, 48.81N, 16.64E, 28-Jan-2016, 1M in *X. obvia*; Mikulov, BV – disused limestone quarry PR Turold, 48.82N, 16.64E, 28-Jan-2016, 1F in *H. pomatia*, 1M & 1F in *X. obvia*; Tasovice, ZN – sand pit, 48.84N, 16.14E, 28-Jan-2016, 2M & 1F in *X. obvia*; Brno, BM – disused limestone quarry Hády, 49.22N, 16.68E, 29-Jan-2016, 3F in *X. obvia*.

## Discussion

We found that the presence of empty snail shells in anthropogenic habitats drives the presence of many empty snail shell adopters in these habitats. However, we have shown that they do not reach similar diversity and abundance as in natural habitats, with only few exceptions (Fig. 3). Thus, we rejected the original hypothesis claiming that the empty terrestrial snail shell adopters reach similar diversity and abundance in anthropogenic and natural habitats. As an illustrative example, let's mention the threatened bombyliid dipteran, *S. aethiops*, which we and others found only in the best-preserved steppic reserves within the study area (Čelechovský, 2015). This species is a parasite of megachilid snail shell-adopting bees, particularly *O. aurulenta* and *Rhodanthidium septemdentatum* (Čelechovský, 2015). Although we found the host species, *O. aurulenta*, to be equally present in both natural and anthropogenic habitats (Table S2), the parasitic dipteran did not manage to follow its host so far.

Furthermore, we tested the hypothesis claiming that the occupancy of shells is constrained by snail species and, thus, that this specialisation contributes to the presence of multiple breeding niches within each sampling site. We have shown that the specialisation for shells made by certain snail species is uncommon, and usually shells of multiple species are occupied as far as they are of similar size that fits the body of the respective insect. Many species did not even discriminate between the three most common shell types and were present both in the large shells of the helicid snails and in the minute shells of *X. obvia* (Fig. 3). Despite strict specialisation was rare, there were insect species that showed significant preferences for certain shell types. For example, *S. fasciata*, the here recorded new member of the Czech entomofauna, occurred preferentially in *X. obvia* shells although we recorded it once also in each *H. pomatia* and *Cepaea* sp. (Fig. 3).

The lower abundance and species richness of insects in anthropogenic habitats were reported repeatedly from many insect assemblages (Pereira *et al.*, 2012; Cardinale, 2014; Geslin *et al.*, 2016; Pereira-Peixoto *et al.*, 2016), but the observations of reverse relationships are also known, particularly in a broad range of disturbance dependent species including, for example, various aculeate Hymenoptera (Cizek *et al.*, 2013; Tropek *et al.*, 2013; Heneberg *et al.*, 2014; Bogusch *et al.*, 2016). Here, we have shown that despite most of the dominant empty snail shell adopters among insects managed to colonise the anthropogenic habitats, they failed to establish there as large populations as in the natural habitats. This points to the limitations of empty snail shells as breeding resources in anthropogenic habitats with regard to the concept of partial habitats (Westrich, 1996), as the species nesting in shells (such as the bees and wasps) likely lack their food resources in anthropogenic habitats and the species that only spend winter in the shells may require both additional food resources as well as breeding resources to be present on site (typically the true bugs).

Several species, to which the concept of partial habitats likely does not apply, displayed a distribution according to the initial hypothesis claiming that the empty terrestrial snail shell adopters reach similar diversity and abundance in anthropogenic and natural habitats. These species, which managed to shift to anthropogenic habitats, consisted predominantly of common flies and beetles that either exploit dead snails or kill living snails (Beaver, 1977; Baalbergen *et al.*, 2014). Below, we will discuss two such specific cases. The first is the snail-killing fly *S. fasciata*. This species appears to expand its distribution range to the north because its first findings have been recorded not only from the Czech Republic (present study) but also from Belgium and the Netherlands (Mortelmans, 2015). It oviposits in the umbilicus of living snails of various species. *Theba pisana* was considered its characteristic host that is native to the Mediterranean and invaded into many other countries. However, other terrestrial snails also may host *S. fasciata* (Knutson *et al.*, 1970; Vala, 1989; Coupland & Barker, 1994; Rozkošný, 2002). Povolný and Groschaft (1959) reported *S. fasciata* from *X. obvia* that was also its core host in the dataset analysed in the present study. The larvae feed on the snail tissues for 2–4 weeks and may putatively kill the snail. Surprisingly, the pupation is thought to take place outside the snail shell and puparia are reported to be found on or in the soil. The pupation stage lasts for 3–5 weeks (Vala, 1989). The adults are active in September; however, isolated records of adults are known year-around (Vala, 1989; Coupland & Barker, 1994). This life cycle is thought to be linked to the life cycle of their main hosts, *Theba* spp. and *Cernuella* spp., as these snails oviposit in the autumn and die shortly after (Coupland & Barker, 1994). Although it was reported to pupate on or in the soil, here we report this fly as abundantly reared from the shells themselves. All the shell material analysed in the present study was collected in the winter period. Although, we did not open the shells but allowed the insects to finish their development and emerge. Thus, we cannot distinguish between the adult individuals that just used the shell as their winter shelter and those that were breeding there. The abundance of *S. fasciata* was very high in the examined material from Hungary and South Moravia. Thus, it is likely that the *X. obvia* shells are used for breeding itself instead of hosting couple remaining adult individuals that otherwise occur very rarely outside the September and October as stated above. Opening of the *X. obvia* shells collected in winter is needed to corroborate this speculation.

The second example of a species, for which the snails are the only breeding and food sources, is the beetle *D. concolor*. The predation by drilids is considered common at some sites throughout Europe. We reared five individuals of this species from *Cepaea* sp. (3), *E. strigella* (1), and *F. fruticum* (1); only one individual originated from an anthropogenic site, which was a long-time

disused limestone quarry in the middle of a large well-preserved karstic area (Table S2). Larvae of *D. concolor* are predators of helicid and clausiliid snails (Baronio, 1974; Schilthuizen *et al*., 1994). The drilid larvae form oval holes in the shells through which they enter in, devour the living snail, moult inside the shell, leave the shell and search actively for a couple of additional snails over the course of 2 years until their metamorphosis (Schilthuizen *et al*., 1994; Baalbergen *et al*., 2016). In the autumn and winter, the larva becomes twice in its life a pseudopupa and enters hibernation. A full-fed drilid larva pupates in the spring. The males are winged. The females are larviform and live mostly hidden inside the shells. Adults of both sexes are, however, short-lived and not expected to overwinter in the shells (Bocák *et al*., 2010). We did not rear any larvae, only five adults (four females and one male). All identified individuals belonged to *D. concolor*. Thus, in the study region, this species occupies the xerothermic sites similar to those occupied by *D. flavescens* in Western Europe although the Western European populations of *D. concolor* appear to be centred more around wet and shady habitats.[2] The reasons why this species did not manage to colonise the anthropogenic sites that are rich in snails remain unclear.

## Conclusions

We provided a conclusive evidence on the presence of species-rich insect assemblages in empty shells of terrestrial snails across a broad range of habitats. Terrestrial snail shells can be considered partial resources according to Westrich (1996), and as such, they are increasingly available in anthropogenic habitats, which is caused by the expansion of multiple snail species to various anthropogenic landscapes in the study area and worldwide. However, we show that this increased availability of snail shells is insufficient for the transition of all species of snail shell adopters from natural to anthropogenic habitats. The avoidance of anthropogenic habitats among snail shell adopters was particularly prominent in species that use them only as a winter retreat but which require additional feeding and breeding resources, such as the true bugs. The availability of snail shells is thus a pre-requisite of the presence of specialised snail shell adopters but is not necessarily sufficient to establish their presence in the respective habitat.

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## Authors' contributions

PBo and PH conceived the ideas and designed methodology; PBo, AA, LH, and PH collected the materials; PBo, JR, PBa, OK, PP, PJ, and KH identified the materials; PH analysed the data and wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## Data accessibility

Essential supporting information is provided as supplementary materials (ESM1 and ESM2).

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** List of sampling sites, including sampling dates, coordinates of the sampling sites and distances between sampling sites in each pair.

**Table S2.** List of insect species found, including their Red List status, the person responsible for the species identification, the number of snail shells positive for the respective species in the natural and anthropogenic environments, sampling sites and host snail species.

[2]http://www.coleo-net.de/coleo/texte/drilus.htm

**Table S3.** Characteristics of insect assemblages that were associated with shells of various snail species. The data shown include number of sampling sites positive for the snail species, number of shells examined, number of insect species recorded, number of insect individuals reared, number of insect individuals per shell, dominance, Brillouin's index, equitability, Fisher's alpha and Berger-Parker dominance index.

**Table S4.** Comparison of insect assemblages that were associated with shells of various snail species using Sørensen index, Morisita-Horn index and combined Chao's Sørensen raw (uncorrected for unseen species) abundance-based similarity.

**Appendix S1.** Current knowledge on obligate and facultative snail shell specialists. Sampling and identification of materials. Supplementary References.

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V rámci výzkumu jsme na vybrané lokality s přirozeně se vyskytujícími ulitami umístili ulity barevně označené (červené, případně žluté) od 6 druhů suchozemských plžů. Jednalo se o druhy *Caucasotachea vindobonensis, Fruticicola fruticum, Helix pomatia, Monacha cartusiana, Xerolenta obvia* a *Zebrina detrita*. Lokalit bylo celkem 21 (16 v ČR, 5 v Maďarsku), 13 z nich bylo přírodního (fragmenty stepních travnatých porostů nebo lesních stepí) a 8 (polo)otevřených antropogenního původu (dva vápencové lomy, dvě pískovny, dva silniční náspy, jedna sprašová vrstva v jílovně a výsypka po těžbě hnědého uhlí).

Na každou lokalitu bylo rozmístěno po 50 ulitách od každého druhu plže s výjimkou druhů *F. fruticum* a *M. cartusiana*, kterých bylo vždy po 10 kusech. Ulity byly pokládány na povrch země ve skupinách lineárně za sebou. Umístění proběhlo během zimy a první poloviny jara 2017 před obdobím hnízdění a ulity byly následně sbírány od podzimu 2017 do zimy 2017–2018. Ulity jsme následně vložili do pytlů z netkané textilie a ponechali při venkovních teplotách chráněné před vodou do ledna. V lednu jsme všechny ulity otevřeli, odebrali z nich vzorky pylu a plísní, které jsme poslali na analýzu, zaznamenali si druh plže a strukturu každého hnízda, dospělé včely byly fixovány v ethanolu, juvenilní jedince jsme přemístili do mikrozkumavek, uzavřeli vatovou zátkou a nechali je dokončit vývoj, následně jsme je určili.

Zjistili jsme, že experimentálně umístěné ulity byly v porovnání s těmi přirozeně se vyskytujícími často více obsazeny, tento rozdíl byl nápadnější na antropogenních lokalitách. Zastoupení druhů ve značených a přirozeně se vyskytujících ulitách bylo podobné, druhy se většinou vyskytovaly v obou typech stanovišť (někdy s vyšší abundancí na přírodních lokalitách), některé byly zaznamenány jen na lokalitách přírodních. Z ulitových včel byly zaznamenány druhy *Osmia aurulenta, O. bicolor, O. rufohirta, O. spinulosa* a *Rhodanthidium septemdentatum,* z jejich blanokřídlých parazitů pak kukaččí včela *Stelis odontopyga*, zlatěnky *Chrysura dichroa* a *C. trimaculata* a drvenka *Sapyga quinquepunctata*. Experimentálně jsme potvrdili, že prázdné ulity plžů slouží jako limitující zdroje pro specializované včely a vosy, a to dokonce i na lokalitách, kde se ulity přirozeně vyskytují v hojném množství. Záleží ovšem nejen na samotné přítomnosti ulit, ale také na jejich kvalitě. Dále jsme opět potvrdili, že si jednotlivé druhy včel pro své hnízdění nevybírají ulity podle konkrétních druhů, ale spíše podle typu ulity či její velikosti.

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# Experimental confirmation of empty snail shells as limiting resources for specialized bees and wasps

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ABSTRACT

Observational studies have suggested that the presence of snail shells correlates with the presence of specialized adopters, particularly bees and wasps (Hymenoptera: Aculeata). However, should the empty shells be considered limiting resources once they are present in the respective habitat even if observational studies suggested low ratio of occupied relative to total shells? We performed a manipulative experiment, which consisted of the addition of marked snail shells of six terrestrial species that dominate the open and semi-open central European habitats to 21 sites with naturally present shells. We deployed empty shells for the spring and summer 2017, allowed their inhabitants to undergo a diapause during the follow-up winter period and complete metamorphosis. The specialized bee and wasp species abundantly occupied the provided shells and the occupancy rates were several times higher in experimentally provided shells compared to the naturally present shells. These differences in occupancy rates were higher at anthropogenic compared to natural sites and at sites with more limited availability of naturally present shells. In contrast, the few sites at which the examined resource was superabundant, both the naturally present and the experimentally provided shells were occupied only to a limited extent. The species composition of assemblages that occupied the experimentally provided and naturally present shells were similar, and the differences in species composition between the natural and anthropogenic sites resembled those exhibited in the naturally present shells. In conclusion, we experimentally confirmed that empty snail shells serve as limiting resources for specialized bees and wasps even at sites where the naturally present shells are perceived as abundant.

## 1. Introduction

For bees and wasps, habitat quality reflects two sets of environmental variables, the availability of food and nesting resources. These resources are often located in a form of partial habitats – both need to be available locally but may be spatially segregated within the habitat (Westrich, 1996). The importance of food resources has been repeatedly demonstrated (e.g., Petanidou and Vokou, 1990) and is already reflected in conservation measures, such as agri-environmental schemes that aim to enhance the floral communities. In contrast, the importance and quality of nesting resources is an emerging yet under-researched topic; with most data available only for soil-nesting species (e.g., Cane, 1991; Potts and Willmer, 1997; Srba and Heneberg, 2012). When the requirements of the whole bee community were analyzed together, about 40% of the variation in species abundance and 61% of the variance in the guild structure were explained by the availability of nesting resources (Potts et al., 2005). Therefore, the presence of specific nesting resources drives the community structure of bees. Two factors dominated the nesting resources needed, the availability of bare ground (for soil-nesting species) and the availability of suitable nesting cavities (for cavity-nesting species) (Potts et al., 2005). The suitable nesting cavities may include empty snail shells, abandoned burrows in wood, plant stems, reed stalks, old walls, rock cavities or old nests of mud daubers or gall wasps. Only some cavity nesters are generalists, whereas most of such species represent taxa that are more-or-less strictly specialized only for one type of nesting cavity. Agri-environmental measures that would support the availability of nesting resources within cultural landscapes are absent, despite the successful habitat management requires nesting resources to be supported to allow diverse bee and wasp communities and to support associated pollination services of bees and biocontrol services of wasps. In the present contribution, we focus on bee and wasp species that utilize empty shells of terrestrial snails as their only nesting habitat.

Observational studies revealed that the presence of terrestrial snail

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shells correlates with the presence of specialized adopters, particularly bees and wasps (Hymenoptera: Aculeata). The shell adopters among the aculeates do not represent a monophyletic group; instead, this behavior developed independently in several genera of mason bees from the *Osmiini* and *Anthidiini* tribes, the crabronid *Tachysphex hermia* and several potter and pollen wasps. The bees that nest in snail shells are further parasitized by the cleptoparasitic bee genus *Stelis*, by multiple species of cuckoo wasps, velvet ants of the genus *Tricholabiodes*, sapygid wasps, beetles from the family Cleridae and the bombylid bee flies *Apolysis hesseana* and *Anthrax aethiops* (Gess and Gess, 1999; Čelechovský, 2015; Müller and Mauss, 2016; Müller et al., 2018; Bogusch et al., 2019). Some of these species have colonized anthropogenic habitats where they nest in shells of expanding snail species. These snail species with expanding distribution ranges are represented in the study region by *Xerolenta obvia*, *Cepaea* spp., *Caucasotachea vindobonensis*, *Helix pomatia*, and *Zebrina detrita*.

Previous studies have been only observational; experimental data are absent. Therefore, it remained unclear whether the empty shells can be considered limiting resources once they are present in the respective habitat even if low ratios of occupied relative to total shells were reported (Bogusch et al., 2019). The abundance of live snails may vary dramatically and may reach over 1000 individuals $m^{-2}$ in the grasslands as well as woodlands (Martin and Sommer, 2004; Cernohorsky et al., 2010). In addition, the presence of empty shells is dependent not only on the presence of live snails but also on the pH of the soil and litter. Lower pH values cause empty shells to dissolve relatively quickly, perhaps even in less than one year and may cause that the numbers of available shells differ by over one order of magnitude across the pH gradient of the soil (Cernohorsky et al., 2010). Consistently with the above, nearly all empty shells that we collected in quartz sand pits were usually of poor quality, brittle and frail, whereas empty shells of the same species that were collected at calcareous grasslands were much better preserved (Heneberg, pers. obs.). Therefore, alongside with the presence of shells, the quality of shells may be limiting the nesting of shell-specialized inquilines as well.

Reflecting the absence of experimental data, we deployed a specific form of trap nests that consists of marked empty shells of terrestrial snails. Trap nests have been used for research on bees and wasps in multiple habitats, and consisted mostly of nests made of various stems (e.g., Gathmann et al., 1994) or other cavities, such as the reed galls (e.g., Heneberg et al., 2017b). The deployment of marked shells allows analyzing, whether the examined sites suffer from a lack of the deployed nesting resource even though the habitat provides abundant (or superabundant) food resources. The deployment also allows the comparison of habitats of various parameters and, thus, the identification of preferred habitats independently on the availability of the deployed nesting resource. In contrast, the limitation of this approach stems from possible absence of specialists for the deployed nesting resource if such a resource is naturally absent from the examined site. To reflect the above, in the present study, we avoided the deployment of marked shells to habitats, from which the naturally present empty shells were completely absent.

In the present study, we hypothesized that the empty shells are a limiting nesting resource for specialized aculeate hymenopterans. To test this hypothesis, we performed a manipulative experiment that consisted of the deployment of marked shells to habitats where thousands of shells were naturally present onsite and of the examination whether and to which extent will the specialized aculeate hymenopterans and associated parasitic species occupy the deployed shells.

## 2. Materials and Methods

### 2.1. Study design

One year ahead of the study onset, we collected empty well-preserved shells of adult individuals of the below-specified snail species and allowed their content to emerge at room temperature in order to make sure that no aculeate hymenopterans were present. We deployed the shells to linear transects in 21 open habitats that were distributed across Czechia (16 sites; two in the Pannonian biogeographic region and 14 in the Continental biogeographic region) and in northwestern Hungary (five sites, all in the Pannonian biogeographic region) (Fig. 1A); the list of transects, including their geographical coordinates, is provided in Table S1. The study sites included 13 natural sites (fragments of steppic grasslands or forest steppes) and eight (semi-) open habitats of anthropogenic origin (two limestone quarries, two sand pits, two roadside verges, a loessic stratum in a claypit, and a lignite open cast mine spoil heap; all sites except the roadside verges were subject to natural vegetation succession). The information on the species composition of empty shells that were naturally present on the soil surface of the examined habitats was available for 15 of the 21 sites and was published previously (Bogusch et al., 2019).

We deployed the following numbers of color-marked shells along each transect: 50 shells of *C. vindobonensis*, 10 shells of *Fruticicola fruticum*, 50 shells of *H. pomatia*, 10 shells of *Monacha cartusiana*, 50 shells of *X. obvia* and 50 shells of *Z. detrita*. In total, we deployed 4620 shells to the soil surface of the transects during the winter and first half of the spring of 2017. We allowed the shells to be exposed at the transects until the end of the vegetation season and collected them from September through December 2017. When searching for the deployed shells, we examined the litter and soil surface in a strip of at least 200 cm in width alongside each transect for the presence of marked shells. We found many of the shells being destroyed; moreover, we had difficulties to find those of the small snail species. Some shells also may have been transferred by the hymenopterans or other organisms outside the surroundings of examined transects. The combination of these resulted in the collection of only 2120 marked shells (45.9% of the deployed number) with the following species-specific ratios of shells found: *C. vindobonensis* 52.8%, *F. fruticum* 47.6%, *H. pomatia* 58.0%, *M. cartusiana* 33.3%, *X. obvia* 36.1%, *Z. detrita* 38.9%. From each transect, we retrieved 101 ± 39 shells (range 30–163 shells).

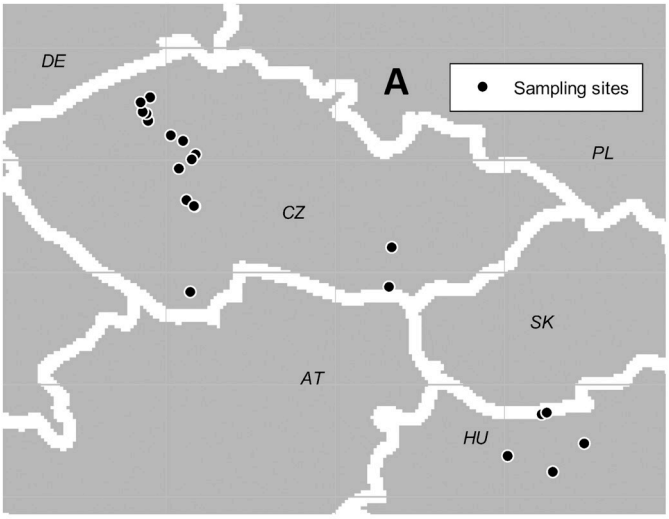
After the collection of the shells, we stored them in ventilated bags made of a nonwoven fabric at the outdoor temperature, protected from water precipitation, until January in order to induce the freeze-induced diapause and enhance the rearing success. In January, we opened all the collected shells, examined their content, recorded the adult bees and moved the juveniles into cotton-plugged Eppendorf microtubes and allowed them to develop when exposed to a daylight cycle at temperatures between 15—23 °C for six months.

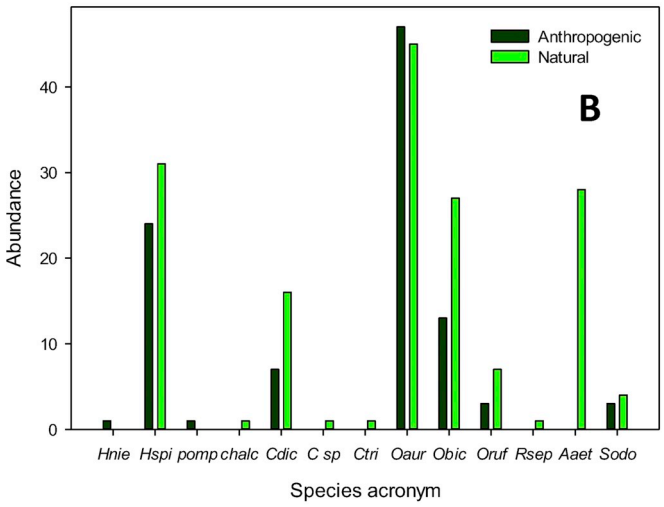
### 2.2. Statistical analyses

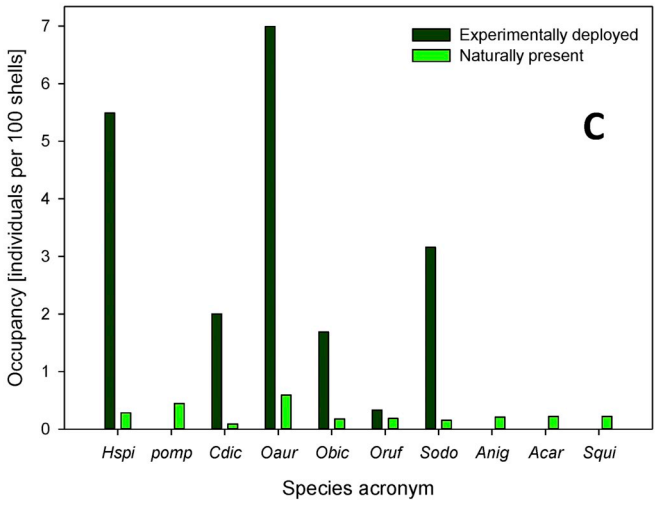
We recorded the number and species of all aculeate hymenopterans and their parasites that were present in the shells. To estimate their species richness, we calculated the Chao-1 estimator, corrected for unseen species. To compare species richness of the analyzed datasets, we calculated the number of shared species, the Sørensen, Bray-Curtis and the combined Chao's Sørensen estimated abundance-based similarity indices. We also calculated total numbers of species and individuals found, and the basic diversity indices including the dominance (D = 1 – Simpson index), equitability, Fisher's alpha and Berger-Parker dominance indices. We compared the diversity indices by bootstrapping. We used $\chi^2$ test with Yates correction to analyze the differences in abundance; normality of the distribution of underlying data was tested using Shapiro-Wilk normality test. We performed all the calculations in SigmaPlot 12.0, EstimateS 9.1.0 and PAST 2.14. Data are shown as mean ± SD unless stated otherwise.

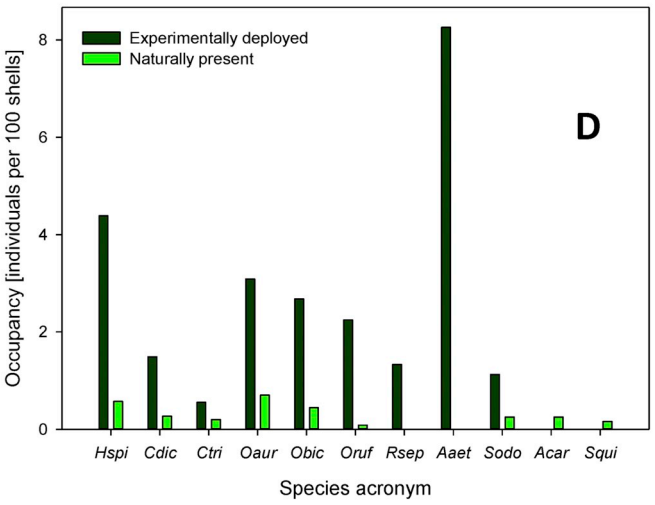
## 3. Results

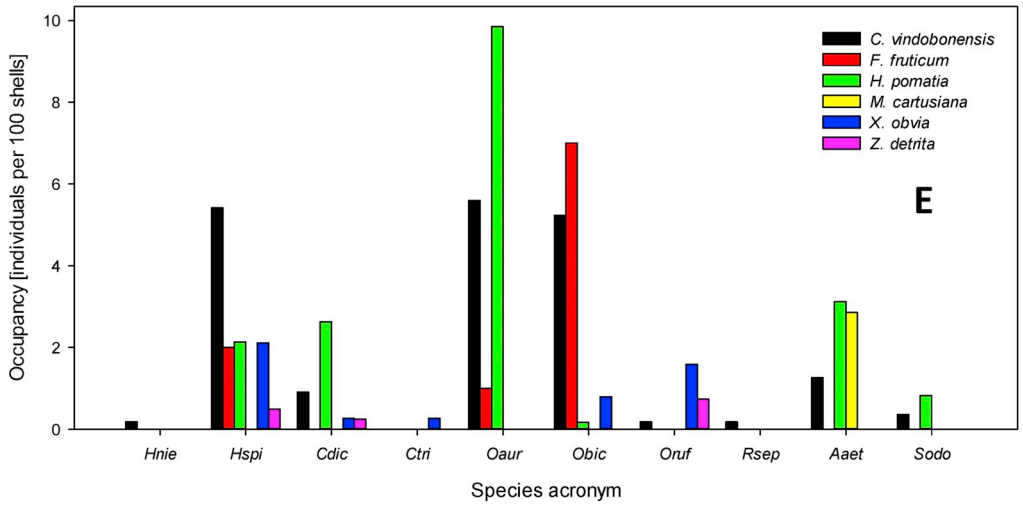
In total, we reared 231 individuals of 10 species of aculeate hymenopterans that we identified to species. In addition, we found one




(caption on next page)

**Fig. 1.** Location of study sites, the numbers of materials examined and the rarefaction curves of reared insects. (A) Location of study sites in the Czech Republic and Hungary. (B) Number of aculeate hymenopterans and their parasites present in shells that were deployed in anthropogenic and natural habitats. (C–D) Differences in the occupancy of experimentally deployed and naturally present shells by aculeate hymenopterans and their parasites in anthropogenic (C) and natural (D) habitats. Data were calculated as numbers of individuals per 100 examined shells from all sites, at which the respective species was present in either experimentally deployed or naturally present shells. (E) Occupancy rates of experimentally deployed shells by aculeate hymenopterans and their parasites. Acronyms of insect species: Hnie: *Hedychrum niemelai*, Hspi: *Hoplosmia spinulosa*, pomp: Pompilidae gen. sp., chalc: Chalcididae gen. sp., Cdic: *Chrysura dichroa*, C sp: *Chrysura* sp., Ctri: *Chrysura trimaculata*, Oaur: *Osmia aurulenta*, Obic: *Osmia bicolor*, Oruf: *Osmia rufohirta*, Rsep: *Rhodanthidium septemdentatum*, Aaet: *Anthrax aethiops*, Sodo: *Stelis odontopyga*, Anig: *Anoplius nigerrimus*, Acar: *Auplopus carbonarius*, and Squi: *Sapyga quinquepunctata*.

**Table 1**
The diversity of assemblages of aculeate hymenopterans and their parasites differed at the anthropogenic and natural sites in experimentally deployed and naturally present shells.

| Group | Anthropogenic habitats | Natural habitats | Experimentally deployed | Naturally present |
|---|---|---|---|---|
| Comparison | Deployed vs. naturally present | Deployed vs. naturally present | Anthropogenic vs. natural habitats | Anthropogenic vs. natural habitats |
| No. of species, group 1 | 8 | 8 | 8 | 9 |
| No. of species, group 2 | 9 | 9 | 8 | 9 |
| Shared species | 7 | 8 | 6 | 7 |
| Sørensen index | 0.823 | 0.941 | 0.750 | 0.777 |
| Chao-Sørensen index, estimated abundance-based, ± SD | 0.985 ± 0.000 | 1.000 ± 0.000 | 0.982 ± 0.000 | 0.999 ± 0.000 |
| Bray-Curtis index | 0.489 | 0.848 | 0.822 | 0.438 |

shell with the representatives of Chalcididae, one shell with unidentified *Chrysura* sp., five aculeate larvae that did not hatch, and 28 individuals of the parasitic bombylid dipteran *A. aethiops*. Detailed list of species retrieved from each examined site is provided in Tables S2–S3.

Concerning the natural and anthropogenic sites, shells that were deployed to either of these habitat types attracted eight species of aculeate hymenopterans, six of which were shared between both datasets. The presence/absence-based Sørensen similarity index reached 0.75 and the abundance-based Bray-Curtis index reached 0.822. However, the Chao's Sørensen estimated abundance-based similarity index reached 0.982 ± 0.000. The dominance was higher at the anthropogenic sites (0.31 vs. 0.23; $p_{\text{bootstrapping}} = 0.02$). The equitability and Fisher alpha were equally low in both habitat types (0.70 vs. 0.78, and 2.05 vs. 1.87, respectively; $p_{\text{bootstrapping}} > 0.05$ for both). The higher value of the Berger-Parker index in anthropogenic habitats (0.47 vs. 0.34; $p_{\text{bootstrapping}} = 0.04$) suggested the higher numerical importance of the most abundant species in these less stabilized habitats. Concerning the comparison of experimentally deployed and naturally present shells, we found that while the assemblages at natural and anthropogenic sites were highly similar to one another, the deployed and naturally present shells differed strongly in abundance at the anthropogenic sites as revealed by the Bray-Curtis index that reached only 0.489 as compared to 0.848 for the natural sites (Table 1). Except incidental findings (1 × *Hedychrum niemelai* and 1 × *Anoplius nigerrimus*), all other species were either equally present at sites of the natural and anthropogenic origin (e.g., *Osmia aurulenta* and *Osmia rufohirta*) or were more abundant at sites of the natural origin (e.g., *Osmia bicolor*, *Hoplosmia spinulosa* and *Chrysura dichroa*), with *Chrysura trimaculata*, *Rhodanthidium septemdentatum* and *A. aethiops* being only reared from sites of the natural origin (Fig. 1B).

As the Bray-Curtis index suggested species-specific differences in abundance, we calculated the occupancy rates of the shells for each species found and analyzed separately for the natural and anthropogenic sites. We found that in both habitat types the experimentally deployed shells were occupied by higher numbers of aculeate hymenopterans compared to the naturally present ones. This difference was more prominent at the anthropogenic sites (Fig. 1C–D) and reached one order of magnitude or more for the dominant species *H. spinulosa* (4.39 vs. 0.57 individuals per 100 shells) and *O. rufohirta* (2.25 vs. 0.08) and only slightly less for other bees of the genus *Osmia*, namely *O. aurulenta* (3.09 vs. 0.70) and *O. bicolor* (2.68 vs. 0.44) ($\chi^2$ test with Yates correction $p < 0.01$ each). In addition, *R. septemdentatum* and the parasitic bombylid fly *A. aethiops* were completely absent from the naturally present shells despite they were present in the experimentally deployed shells, with *A. aethiops* being represented by 28 individuals from multiple nests. There was no common species that would be more abundant in the naturally present shells.

Despite large numbers of experimentally deployed and naturally present shells, the hymenopterans occupied only a fraction of them, with numerous findings of nesting bees in only the experimentally deployed or only the naturally present shells. For *O. aurulenta* and *O. bicolor*, the numbers of sites where the experimentally deployed shells were occupied but the naturally present were not exceeded those for which the reverse would be true or those at which both types of shells would be occupied. The presence/absence data were as follows: *O. aurulenta* was present at 8 sites in deployed shells only vs. at 1 site in both shell types vs. at 2 sites in naturally present shells only, and *O. bicolor* was present at 8 sites in deployed shells only vs. at 3 sites in both shell types vs. at 1 site in naturally present shells only (Table 2). We did not find any species that would be identified at a statistically higher number of sites based on the collection of the naturally present shells despite the numbers of collected naturally present shells were always several times higher than the numbers of experimentally deployed shells; the species with the distribution most inclined towards the naturally present shells were *O. rufohirta* (1 vs. 2 vs. 3 sites) and *Auplopus carbonarius* (0 vs. 0 vs. 3 sites). We cannot exclude the effects of the microhabitats or differences between years as the naturally present shells were collected in 2016 but the experimentally deployed ones were collected in 2017. At some sampling sites, such as Bikolpuszta or Brno-Hády, we found six species of hymenopterans and their parasites to occupy the experimentally deployed shells but no species in the naturally present shells. In contrast, at the sampling site Písečný vrch, there were only three species in the deployed shells, all of which were simultaneously present in the naturally present shells. However, the naturally present shells hosted additional five species, some of which (*O. aurulenta* and *H. spinulosa*) were present at a very high abundance and readily occupied experimentally deployed shells at other sites.

Regarding the preferred snail species, the abundantly present aculeate hymenopteran species accepted shells of multiple snail species with the lowest abundances found in the small species. Particularly, *Z. detrita* attracted a very limited spectrum of hymenopterans and all in low abundances, with only *H. spinulosa*, *C. dichroa* and *O. rufohirta* found in experimentally deployed shells of this species (Fig. 1E). In

**Table 2**
Comparison of the proportion of aculeate hymenopterans and their parasites that were present in both experimentally deployed and naturally present shells at the same site, or only in experimentally deployed or naturally present shells in anthropogenic and natural habitats. Shown are the numbers of sampling sites that fulfilled the specified conditions.

| Habitat | Anthropogenic ($n_{paired}$ = 6) | | | Natural ($n_{paired}$ = 9) | | |
|---|---|---|---|---|---|---|
| Species\shell type | Experimentally deployed | Both | Naturally present | Experimentally deployed | Both | Naturally present |
| *Hoplosmia spinulosa* | 1 | 1 | 1 | 4 | 0 | 2 |
| Pompilidae gen. sp. | 0 | 0 | 1 | | | |
| *Chrysura dichroa* | 1 | 1 | 0 | 3 | 1 | 2 |
| *Chrysura* sp. | | | | 0 | 0 | 1 |
| *Chrysura trimaculata* | | | | 1 | 0 | 1 |
| *Osmia aurulenta* | 2 | 1 | 1 | 6 | 0 | 1 |
| *Osmia bicolor* | 3 | 1 | 0 | 5 | 2 | 1 |
| *Osmia rufohirta* | 1 | 1 | 3 | 0 | 1 | 0 |
| *Rhodanthidium septemdentatum* | | | | 1 | 0 | 0 |
| *Anthrax aethiops* | | | | 3 | 0 | 0 |
| *Stelis odontopyga* | 0 | 1 | 0 | 0 | 1 | 1 |
| *Anoplius nigerrimus* | 0 | 0 | 1 | | | |
| *Auplopus carbonarius* | 0 | 0 | 1 | 0 | 0 | 2 |
| *Sapyga quinquepunctata* | 0 | 0 | 1 | 0 | 0 | 1 |

contrast, *X. obvia* hosted a broader spectrum of species, but most of them in low abundance, again except *H. spinulosa* and *O. rufohirta*. The latter species was abundantly present only in these small-sized shells, was completely absent from the shells of adult *H. pomatia* and was only sporadically present in the shells of *C. vindobonensis* (Fig. 1E). The rather thin shells of *M. cartusiana* were avoided at all and we detected only two such shells with *A. aethiops* but unknown host species. In contrast, the shells of *H. pomatia*, *F. fruticum* and *C. vindobonensis* were abundantly occupied, with *O. aurulenta*, and the parasites *S. odontopyga*, *C. dichroa* and *A. aethiops* being more abundant in *H. pomatia* shells. The *C. vindobonensis* shells, together with the *F. fruticum* shells, were preferred by *O. bicolor* and hosted exclusively the less abundant species *R. septemdentatum* and *H. niemelai*.

Some of the nests were mixed. For example, the only nest of *O. rufohirta* in other than small-sized snail species was a mixed nest with *O. bicolor* (nest from the sampling site Zájezd, in order from the plug: *O. rufohirta* M, *O. bicolor* M, *O. bicolor* M). The parasitized nests included those of *O. aurulenta* that were parasitized by *C. dichroa* (nest from the sampling site Zájezd, with five chambers, in order from the plug: *O. aurulenta* M, *C. dichroa* M, *O. aurulenta* M, *O. aurulenta* F, *O. aurulenta* F; nest from the sampling site Kamýk, with seven chambers, in order from the plug: *O. aurulenta*, *C. dichroa*, *C. dichroa*, *O. aurulenta*, *O. aurulenta*, *C. dichroa*, *O. aurulenta*; nest from the sampling site Patokryje, with four chambers, in order from the plug: *O. aurulenta*, *C. dichroa*, *C. dichroa*, *O. aurulenta*; nest from the sampling site Raná, with eight chambers, in order from the plug: *C. dichroa*, *O. aurulenta*, *O. aurulenta*, *C. dichroa*, *O. aurulenta*, *O. aurulenta*, *O. aurulenta*, *O. aurulenta*; and other less conclusive nest arrangements). One nest of *O. bicolor* was also parasitized by *C. dichroa* (nest from the sampling site Prokopské údolí, with two chambers, in order from the plug: *C. dichroa* F, *O. bicolor* M). One nest of *H. spinulosa* was also parasitized by *C. dichroa* (nest from the sampling site Raná, with three chambers, containing 2× *C. dichroa* and 1× *H. spinulosa*). One nest of *H. spinulosa* was also parasitized by *S. odontopyga* (nest from the sampling site Radovesická výsypka, with three chambers, in order from the plug: *H. spinulosa*, *S. odontopyga*, *S. odontopyga*; nest from the sampling site Radovesická výsypka, with two chambers, in order from the plug: *H. spinulosa*, *S. odontopyga*). The nests with *A. aethiops* did not contain any host species larvae.

## 4. Discussion

We experimentally confirmed that empty snail shells serve as limiting resources for specialized bees and wasps even at sites where the naturally present shells are abundant. Therefore, the empty shells limit the ability of the specialized bee and wasp species to nest in the respective habitats. The concept of nesting limitation by the availability and quality of empty shells is similar to the concept of limitation by other resources that are needed by the bees and wasps – the food resources and various other nesting resources. If empty snail shells are absent, the specialized bee and wasp species cannot be present. Moreover, the experimentally deployed shells were adopted even at sites where naturally present shells were superabundant. It means that the deployed shells were of higher perceived quality, perhaps because they were empty, without any ants colonies or spiders present at a time of addition and because they were clean, without any decaying matter inside of them. The limiting role of the presence of shells at sites where the naturally present shells are abundant was unexpected prior the present study was performed. It resembles the situation with other cavity adopters, which are often limited by various physical characteristics of the cavities and their surroundings. This dependence is particularly known for cavity adopters among birds, which are known to reflect not only the number of available cavities (Newton, 1994; Gibbons and Lindenmayer, 2002; Heinsohn et al., 2003; Cockle et al., 2010) but also their quality (Cockle et al., 2008). The limited availability of cavities is characteristic particularly for human-altered habitats (Wiebe, 2011) whereas the cavities are not considered limiting nesting resources when being superabundant as are the cavities in mature forests (Wesołowski, 2007). Cavity adopters among birds leave many, up to 97%, of available cavities in natural forests unoccupied, but many (but not all) of their populations increase after the provisioning of additional high-quality cavities. In line with the present study, Cockle et al. (2008) found a low occupancy of naturally present cavities, which was in contrast to a much higher occupancy of experimentally deployed cavities of uniform size (nest boxes), concluding that this is because high quality cavities are rare and may limit populations of cavity adopters. Similar data were also obtained by Lõhmus and Remm (2005). Some others studies which did not report increases in abundance reported a switch from naturally present to experimentally deployed cavities (Drent, 1984; Gauthier and Smith, 1987). Regarding cavity adopters among insects, previous evidence on cavities as limiting nesting resources is restricted to only a few studies. However, previous studies that focused on cavities in common reed *Phragmites australis* galls provided similar conclusions, suggesting that the reed galls comprised a limiting nesting resource for obligate reed specialists within reed stands. This effect was more prominent at sites with human-altered (mown) reed beds, which led to a sharp decrease in a number of naturally available galls, but the species richness and abundance of aculeate hymenopterans nesting in the trap nests dropped dramatically when examined in galls that were deployed at sites distant from any reed stands (Heneberg et al., 2017b).

In the present study, we found the experimentally deployed shells to be occupied by proportionally more individuals compared to the naturally present ones. This difference was very robust and was most prominent at anthropogenic sites but present to a lower extent at natural steppic fragments too. Because we did not expect the experimentally deployed shells to be occupied more than the naturally present shells (as all the deployed shells were once present at some of the study sites), the experiment was not designed in a way that would allow tracking reasons for this observation. One possible explanation could stem from the way of collection of the shells. The experimentally deployed shells included those that were not visible on the surface but were covered with grass, litter or upper soil strata. In contrast, the naturally present shells were collected based on their visibility at the soil surface or under the grass, twigs and leaves; however, we did not search for them anywhere deeper in the litter or in the soil itself. This may have caused that the species whose shells were hidden in the litter or in burrows of vertebrates escaped our attention and biased the total numbers downwards. The other explanation could stem from a better quality of experimentally deployed shells as we did not deploy those that were partially or fully broken, thin-shelled, crushy, etc. Third, all the experimentally deployed shells were dry, free of any arthropods that nest in decaying snail bodies. We do not know how many of the shells that were collected as naturally present represented the snails that died in the respective year. However, based on high abundances of snail predators and specialists for dead snails, both reared from naturally present shells, it is possible that these shells may be considered as unsuitable by the aculeate hymenopterans until the snail body is completely decomposed or the respective snail may die only after the nesting period of aculeate hymenopterans. We assume that these freshly dead snails may comprise a large proportion of the collected shells, particularly at sites with neutral or acidophilous pH reaction of the soil where the shells decay quickly and the most of those that are present are not older than one or a few years (Cernohorsky et al., 2010).

Importantly, the comparison of the present study on experimentally deployed shells to previously published data concerning the naturally present shells (Bogusch et al., 2019) revealed that the spectrum of species found was overlapping only when considering large datasets like all the examined sites of anthropogenic or natural origin but not the individual sampling sites. At the level of individual sampling sites, the two methods often identified different species despite we convincingly showed that they were capable to identify nearly identical species assemblages. This means that the numbers of collected experimentally deployed shells (30–163 shells per site) or naturally present shells (~500 shells per site) are insufficient to provide the full information on spectra of specialized shell adopters that are present onsite. However, collecting higher numbers of shells is often difficult because of their hidden positions, particularly where they are not superabundant. The pan trap experiments are thus likely more representative at least for species that are attracted to the traditionally used bright colors (Heneberg and Bogusch, 2014), which was corroborated by pan trapping that was previously conducted at sampling sites Kvíc and Louny (Heneberg et al., 2017a).

Concerning the specialization of the examined species, we confirmed previous observational data on the specialization of *O. rufohirta* for *X. obvia* and other small-sized shells, which was suggested already by Bellmann (1981). Other than that, the dominant species showed little specialization with the most of them occupying readily the large shells made by adult *H. pomatia* and intermediate shells of *C. vindobonensis*. This is in contrast to some of the previous studies. For example, Müller (2018) in his recent comprehensive review of the *Hoplosmia* (sub)genus claimed *H. spinulosa* to be a specialist for "empty snail shells of small to medium size". However, in our manipulative experiment, *H. spinulosa* readily accepted *H. pomatia* shells in proportionally same numbers as those of *X. obvia* and four times more often than those of *Z. detrita* with the numbers that were biased even more towards *H. pomatia* reported by the matching observational study (Bogusch et al., 2019). Another dominant species, *O. aurulenta*, was previously reported to "usually colonize medium-sized to large shells" (Müller et al., 2018). We found this species in these types of shells only with the shells of *H. pomatia* being preferred over those of *C. vindobonensis* in a ratio 9.9:5.6 despite the distribution among these two host shell species was roughly equal when using the observational data from the same region (Bogusch et al., 2019).

Concerning the conservation of the species found, the present study provided the first experimental evidence on the importance of high-quality shells as partial habitats that allow the presence of specialized aculeate hymenopterans. We show that empty shells are special nesting resources that can limit the breeding densities of the specialized species even if "some" shells are present on-site. We still do not know what the parameters that denote the high- and low-quality shells are. Nevertheless, we assume that at least a part of the perceived quality is related to the content of the shell where the remains of the snail body should be absent or removable. In addition, the shell itself should be resistant to physical damage. The physical damage resistance may be difficult to secure in areas with neutral or acidic soils and which may also be more difficult to achieve in areas affected by acid rains (Cadée, 1999) and reflecting the decline in snail densities at calcium-poor (but not at calcium-rich) soils during last decades (Graveland et al., 1994; Graveland and van der Wal, 1996).

The manual addition of shells to habitats, where they are absent, would contribute a little. Although it would be definitively useful at a small-scale, it would resemble similar only temporarily- and locally-effective attempts like the construction of "bee houses" with various cavities for trap-nesting species. These types of management work when the pollinator should be attracted to the respective orchard or a specific garden but does not work at the landscape scale. From the nature conservation point of view, the main conservation measure should be the support of local snail populations by more stringent conservation of non-arable areas with loose vegetation within and around orchards, typically at south-exposed slopes or at soils with low water holding capacity/cation exchange capacity. More stringent protection of such areas would support the presence of vital snail populations that generate the sufficient amounts of empty shells for shell inquilines. We have shown that the specialized shell adopters among hymenopterans require the presence of shells of yet unknown specific properties, which likely reflect the shell size, shell hardness, the absence of other shell inquilines and the absence of decaying wet matter inside the shell. In addition, the complete absence of shells from sites with abundant floral resources completely prevents the presence of shell adopters. Some of these species adapted to cultural landscapes and occupy roadside verges and mines, but others have more specific habitat demands and survive only in areas where the loose vegetation is present naturally or developed for decades since the last major disturbance, although the low-intensity pressure of large herbivores could be beneficial for the sustainability of many of these habitats. The shells remain onsite for a long time only at areas with neutral or alkali soils; therefore, further research should also address the question, whether soil liming can contribute to better preservation of empty shells and whether it could be used as a conservation measure at orchards with sandy soils.

Concluded, the present study identified empty snail shells as previously undervalued limiting nesting resource, the addition of which can increase the populations of specialized aculeate hymenopterans even at sites where empty shells are naturally present.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2019.105640.

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**5.3 Bogusch P., Hlaváčková L., Šilhán K., Horsák M. 2020a: Long-term changes of steppe-associated wild bees differ between shell-nesting and ground-nesting species. *Journal of Insect Conservation* 24 (3), 513–523. https://doi.org/10.1007/s10841-020-00232-4.**

Většina druhů včel a vos obývající stepi patří dle posledních červených seznamů mezi druhy nejohroženější, mizící postupně z fauny střední Evropy. Ohrožené jsou především druhy hnízdící v zemi. Naopak druhy hnízdící v prázdných ulitách se začaly šířit více než v minulosti a v aktuálních červených seznamech povětšinou nefigurují (Straka & Bogusch 2017). Doposud však neexistovala žádná studie, která by se změnami šíření těchto stepních druhů zabývala.

My jsme v našem výzkumu shromáždili veškerá dostupná publikovaná i nepublikovaná faunistická data i ochranářská opatření. Cílem bylo zjistit, zda jsou druhy hnízdící v ulitách v současné době běžnější než v minulosti, porovnat rozšíření těchto druhů včel s druhy hnízdícími v zemi (rod *Eucera*) a definovat hlavní faktory, které by mohly vysvětlit možné rozdíly.

Shromáždili jsme informace o všech šesti druzích včel hnízdících v ulitách v ČR (*Osmia andrenoides*, *O. aurulenta*, *O. bicolor*, *O. rufohirta*, *O. spinulosa*, *Rhodanthidium septemdentatum*), třech v ulitách parazitujících zlatěnkách (*Chrysura cuprea*, *C. dichroa*, *C. trimaculata*) a kukaččí včele *Stelis odontopyga* parazitující v hnízdech *O. spinulosa*. Ze skupiny blanokřídlých hnízdících v zemi a vyskytujících se na stepních biotopech jsme k porovnávání vybrali druhy *Eucera alticincta* (Lepeletier, 1841), *Eucera dentata* (Germar, 1839), *Eucera fulvescens* (Giraud, 1863), *Eucera interrupta* Baer, 1850, *Eucera longicornis* (Linnaeus, 1758), *Eucera malvae* (Rossi, 1790), *Eucera nigrescens* Pérez, 1879 a *Eucera pollinosa* Smith, 1854. Seskupili jsme záznamy sledovaných druhů získané především v muzeích a osobních sbírkách v rámci České republiky. K jednotlivým druhům jsme vyhledali GPS souřadnice jejich nálezů. Data byla následně rozdělena do dvou skupin (nálezy získané před a po roce 1990) a společně s klimatickými daty počítačově zpracována.

Výsledky ukázaly, že druhy hnízdící v prázdných ulitách (s jedinou výjimkou, kterou tvoří druh *O. andrenoides*) opravdu zvětšily velikosti svých populací a rozšířily se na více lokalit oproti minulosti. Oproti tomu u včel hnízdících v zemi došlo k opačnému vývoji, tedy k poklesu jejich početnosti a zmenšení areálů jejich rozšíření. Klimatická data navíc naznačila, že druhy včel hnízdící v prázdných ulitách plžů se šíří směrem k vyšším nadmořským výškám a do míst s nižšími průměrnými červencovými teplotami. U druhů hnízdících v zemi se vliv klimatických podmínek ve většině případů nijak významně neprokázal.

Naše výsledky tedy naznačují, že druhy hnízdící v ulitách jsou schopny kolonizovat nové oblasti ve vyšších nadmořských výškách a/nebo s chladnějšími letními teplotami, na rozdíl od včel hnízdících v zemi. Ve většině případů se navíc zdá, že ulitové druhy vytvořily silnější populace než dříve. Včely hnízdící v prázdných ulitách tak pozitivně reagovaly na aktuální stav ve svých stanovištích a vytvořily v nich silné populace. Je také zajímavé, že druhy preferující otevřené nezarostlé lokality (tj. *O. rufohirta, O. spinulosa* a *C. cuprea*) se rozšířily více než druhy preferující travnaté a křovinaté svahy (tj. *O. aurulenta, O. bicolor* a *C. dichroa*). Tato skutečnost může souviset i se schopností několika druhů usazovat se na antropogenních lokalitách, jako jsou železniční náspy a krajnice silnic.

Důvodem šíření druhů hnízdících v ulitách a úbytku druhů hnízdících v zemi je nepříznivý management v průběhu 20. století vedoucí k přeměně stepních formací v křovinná stanoviště, dále přetváření těchto lokalit v lesní plantáže nebo pole a s těmito změnami související vzrůstající počet dostupných plžích ulit jakožto vhodných hnízdních zdrojů pro ulitové druhy. Mnoha druhům plžů totiž zarostlé biotopy vyhovují mnohem více, krom toho někteří plži začali více obsazovat také antropogenní lokality, jako jsou výsypky, bývalé vojenské prostory, náspy silnic a železnic apod. Změny lokalit jsou dále spojeny s úplnou absencí holých půdních ploch či přeměnou na půdu tvrdou, nevhodnou k hnízdění, což vedlo k úbytku hnízdních zdrojů pro druhy hnízdící v zemi. Vhodným managementem by se však zřejmě dalo zajistit, aby byly stepní lokality vhodné pro hnízdění nejen ulitových druhů, ale i druhů hnízdících v zemi.

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ORIGINAL PAPER

# Long-term changes of steppe-associated wild bees differ between shell-nesting and ground-nesting species

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## Abstract

Bees and wasps inhabiting steppe formations are, according to recent red lists, among the most endangered species, quickly disappearing from local faunas of central European countries. Several species, which are specialised nesters in empty gastropod shells, show the opposite pattern. Based on their distribution maps, we found that these species are recently more common and widespread than in the past. In contrast, the bee species nesting in the ground showed clearly the opposite result. They are much rarer now than in the past. Climatic data suggest that the bees nesting in gastropod shells have expanded towards higher altitudes and/or areas of lower mean July temperature. The main reasons of these distributional shifts are (i) unfavourable nature conservation management practices during the twentieth century, resulting in the successional shifts of steppe formations towards shrubs and mesic grasslands, (ii) human-driven change of many sites towards wood plantations or crop fields, and (iii) the increase of the number of available gastropod shells (i.e. nesting resources for shell-nesting bees) in steppe habitats as the response to the mentioned successional changes.

**Keywords** Distribution · Steppe habitat · Reduction · Conservation · *Eucera* · *Osmia* · *Chrysura*

## Introduction

Global changes in biotopes during approximately the last hundred years have had a notable effect on the populations of nearly all plant and animal groups (Van Swaay et al. 2006; Anderson 2008; Čížková et al. 2013). Populations of various species have been negatively influenced across multiple spatial scales. Degradation or even destruction of the whole biotopes have resulted in local or global extinction of many specialized species (e.g. the butterfly *Chazara briseis* (Linnaeus, 1764), see Johannesen et al. 1997; Kadlec et al. 2010). In the case of bees, the European Red List of Bees (Nieto et al. 2014) shows that 9.2% of European species are extinct or endangered, which is mostly caused by significant changes of the habitats. Regional red-lists show very similar results with usually even higher proportions of endangered species. For example, 86 species (14.5% of all 596 species recorded) of the bee species reported from the Czech Republic are currently Regionally Extinct (RE), and other 190 species (31.8%) are considered Critically Endangered (CR), Endangered (EN), Vulnerable (VU), and Near Threatened (NT) (Straka and Bogusch 2017a).

Azonal open-country habitats of the Central-European temperate are among the most endangered biotopes, usually due to their scattered distribution and small areas. Steppes on southern and south-western hill slopes in warm regions of the Czech Republic (thermophyticum sensu Skalický 1988) are unique biotopes across the whole of Europe and represent the most important remnants of the communities that have been widespread across central European lowlands since the last glacial period (Ellenberg 1988; Čížek et al. 2012; Pokorný et al. 2015; Divíšek et al. 2020). These habitats host several steppe species of plants and animals characteristic for glacial loess steppes during the cold intervals of the Pleistocene (Horsák et al. 2015). Human activities have maintained their occurrence since the Neolithic, keeping the landscape open (Ložek 1964; Horsák et al. 2010; Pokorný et al. 2015). The occurrence of many of them is associated with the loess areas in Central Europe, while others prefer rocky limestone sites (Ložek 1964; Macek et al. 2010).

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However, substantial changes of the landscape over the last two or three centuries have reduced these steppe areas to only very small refugial fragments surrounded by the cultural landscape of fields, human settlements and forests. Also, the afforestation by humans had the key importance for the distribution of open habitats and their characteristic species. It made the populations to become more and more isolated, likely making the dispersal even among nearby sites difficult (Hensen et al. 2010; Lindborg et al. 2012). However, recent plant and insect surveys showed that both groups contain species that have sufficiently high dispersal abilities to colonise new sites (Tyrner 2005; Laštůvka 2009; Lindner et al. 2010). Therefore, it seems that the isolation of suitable sites is not the main reason for the decline of these steppe species, as usually suggested. Thus, successional changes in the structure and composition of vegetation might rather be an alternative explanation for these species declines. This assumption is suggested by often not very precisely designed or applied conservation management that mostly focuses only on the species conservation but not on the entire habitats. Many of these steppe sites have turned to forest-steppes, shrubby slopes or became overgrown by meadow grass communities during the twentieth century (Heneberg et al. 2013; Tropek et al. 2013).

Bees and wasps (belonging to the monophyletic Aculeata group) are usually associated with open habitat conditions. A high proportion of the Central-European Aculeata fauna is specialized to live in steppe habitats, while other habitat generalists occur in any open and warm habitats, including steppes (see Westrich 1989, 2018; Blösch 2000; Wiśniowski 2009, 2014; Macek et al. 2010; Falk and Lewington 2015; Scheuchl and Willner 2016). Thus, steppe biotopes can be inhabited by most of the local faunas of bees and wasps and may serve as the hotspots of the biodiversity for these species. The recent red lists show an alarming scenario that exactly the species associated with steppes are usually those being most endangered or even regionally extinct. For example, 92 out of 136 bee species highly preferring steppe habitats are included in the Czech Red List of Bees (Straka and Bogusch 2017a), which means that more than two-thirds of bees disappeared from their habitats or are close this situation. Including regionally extinct, critically endangered and endangered species, the proportion of species depending on steppe biotopes is the highest according to the red-lists (Amiet 1994; Westrich et al. 2011; Bogusch and Straka 2017a, b; Straka and Bogusch 2017a, b; for detailed results see Table 1). Such a loss of only some species can be considered a consequence of the changes in steppe biotopes.

Surprising, on the other hand, several species of bees bound to steppe biotopes have recently become more widespread than they had in the past. It is true, however, for several species nesting in empty gastropod shells (Straka and Bogusch 2017a). While ca 30 species in Europe nest in shells (Müller 1994; Müller et al. 2018), only six of them also occur in the Czech Republic (Bellmann 1981; Macek et al. 2010; Müller 2018) These bees are unique in the fact that they make their nests only in empty gastropod shells and use other types of cavities only very sporadically or not at all. Furthermore, all these species highly prefer steppe biotopes. Their development is also connected with their species-specific parasites found only in their nests (genera *Chrysura* Dahlbom, 1845 (Hymenoptera: Chrysididae) and *Stelis* Panzer, 1806 (Hymenoptera: Megachilidae), see Macek et al. 2010). However, there is no study quantifying modern changes in the distribution and frequency of these shell nesting species and possible causes of these changes.

In this study, we assembled all the available faunistic data from published and unpublished sources as well as conservation measures to (i) explore whether the species nesting in empty gastropod shells have recently become more common and widespread than in the past, (ii) compare their distributional data with a group of species nesting in the bare ground (genus *Eucera* Scopoli, 1770), and (iii) define the main factors that might explain possible differences. Bees of the genus *Eucera* were selected because (i) there is enough data on their occurrence in the Czech Republic; (ii) in the past they were recorded from the same or nearby localities as the shell-nesting species; (iii) they share the flight season with most of the shell-nesting species; and (iv) there is only a very limited number of ecologically similar species

**Table 1** Regionally extinct species in the Czech Republic with the number of records in the past; the last known site and year of the occurrence is also shown

| Species | Distribution | Last record |
|---|---|---|
| *Eucera caspica* | One record | Čejč; 1940 |
| *Eucera cineraria* | Two localities in the southeast part of the country | Pouzdřany; 1940 |
| *Eucera clypeata* | Two localities in the southeast part of the country | Mutěnice; 1943 |
| *Eucera hungarica* | One record in Bohemia and eight localities in the southeast part of the country | Prostějov; 1961 |
| *Eucera nana* | One record | Pouzdřany; 1937 |
| *Eucera salicariae* | Three localities in the southeast part of the country | Dolní Věstonice; 1976 |
| *Eucera seminuda* | Five localities in the southeast part of the country, very close to one another | Mutěnice; 1943 |

classified in the same family Megachilidae as all the shell-nesting species (most of them nest in cavities, if they nest in ground, they are usually regionally extinct).

## Materials and methods

We assembled data about all six shell nesting species in the Czech Republic, i.e. *Osmia andrenoides* Spinola, 1808, *Osmia aurulenta* (Panzer, 1799), *Osmia bicolor* (Schrank, 1781), *Osmia rufohirta* Latreille, 1811, *Osmia spinulosa* (Kirby, 1802), and *Rhodanthidium septemdentatum* (Latreille, 1809), three cuckoo wasps parasitising in their nests, i.e. *Chrysura cuprea* (Rossi, 1790), *Chrysura dichroa* (Dahlbom, 1854), and *Chrysura trimaculata* (Förster, 1853), and the cuckoo bee *Stelis odontopyga* Noskiewicz, 1926, a nest cleptoparasite of *O. spinulosa*. As the group of species nesting in the bare ground and specialised on steppe habitats in the Czech Republic, we collected all faunistic data for *Eucera alticincta* (Lepeletier, 1841), *Eucera dentata* (Germar, 1839), *Eucera fulvescens* (Giraud, 1863), *Eucera interrupta* Baer, 1850, *Eucera longicornis* (Linnaeus, 1758), *Eucera malvae* (Rossi, 1790), *Eucera nigrescens* Pérez, 1879, and *Eucera pollinosa* Smith, 1854. We did not tackle other species of this genus (i.e. *Eucera caspica* Morawitz, 1873, *Eucera cineraria* Eversmann, 1852, *Eucera clypeata* Erichson, 1835, *Eucera hungarica* (Friese, 1895), *Eucera nana* (Morawitz, 1874), *Eucera salicariae* (Lepeletier, 1841), and *Eucera seminuda* Brullé, 1832), that had been recorded only in a single or several localities in the past and had recently become regionally extinct according to the Check-list (Bogusch et al. 2007) and Red-list of bees (Straka and Bogusch 2017a).

We databased all the records of the selected species from the museums and private collections in the Czech Republic. It provided the majority of all known records of these species from the Czech Republic. The collections from which the records originate are listed in Table 2. Several data were obtained from published sources (usually faunistic notes from Czech journals), BioLib web server and datasets collected by the Czech Agency of Nature Conservation (AOPK ČR). We sorted all the data to the species and searched for the GPS coordinates, if not available with the records. The data were divided into two groups: (i) records before (called as "old" or "past" in the figures) and (ii) after (called as "new" or "recent" in the figures) the year 1990. These two datasets are also best suitable for the comparison because most of the distribution data were collected between 1930–1955 (when many hymenopterists were active in former Czechoslovakia) and 1995–2017 when the situation was very similar. The intensity of the research on bees and wasps in both periods was similar, with a similar number of collectors. They have been using preferably netting and sweeping as the main methods, while usually yellow pan traps become additionally used only recently. Steppes were the most preferred habitats by entomologists in the past. Therefore, it is possible to compare records from the past and present as the data are less biased by the intensity of studies than, for example, those from wetlands and forests.

The spatial distribution of all species in both periods (old and new) was processed in the ArcGIS ver. 10.2 software (ESRI 2012). The GPS coordinates presented in the World Geodetic System 1984 (WGS84) were transformed into the point-type shapefile layer. The density and clustering of all localities were presented as a spatial distribution of the Kernel density estimation (KDE; Silverman 1986). In the analyses of spatial data, the KDE function is commonly used for the smoothing purposes that are based in the counting of the points in the region (kernel) centred at the position (pixel or grid point centre) where the estimation is made

**Table 2** List of the studied collections

| Abb | Collection name |
|---|---|
| APPC | Antonín Přidal, private collection |
| DBPC | Daniel Benda, private collection |
| DVPC | Dušan Vepřek, private collection |
| HKMC | Museum of east Bohemia, Hradec Králové (curator Bohuslav Mocek) |
| JGPC | Jaroslav Gahai, private collection |
| JSPC | Jakub Straka, private collection |
| LBPC | Lukáš Blažej, private collection |
| MHPC | Marek Halada, private collection |
| MMBC | Moravian Museum, Brno (curator Igor Malenovský) |
| MRPC | Martin Říha, private collection |
| MSBC | Museum of south Bohemia, České Budějovice (curator Zdeněk Kletečka) |
| NMPC | National Museum, Praha (curator Jan Macek) |
| PBPC | Petr Bogusch, private collection |
| PTPC | Pavel Tyrner, private collection |

(O'Sullivan and Unwin 2010). The area corresponding to the extent of occurrence (EOO—area contained within the shortest continuous imaginary boundary that can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon, excluding cases of vagrancy) was measured as a standard minimum convex polygon (Gaston 1991; IUCN 2012; Syfert et al. 2014) in the GIS software environment. In the following step, the population density was calculated based on the EOO area and the number of localities. Climatic variables were adopted from the Atlas of climate of the Czech Republic (Tolasz 2007), having a resolution of 1 $km^2$. Mean annual, July and January air temperatures, and annual precipitation sums were obtained based on the geographic coordinates using the ArcGIS 8.3 program (www.esri.com) and a digital elevation model and the climatic maps referred above. Differences in elevation and mean July temperature values between old and modern records of the ground-nesting and the shell-nesting bee species were tested using the generalized estimating equations (GEE) with a Gaussian error structure. GEE is an extension of the generalized linear models for situations when measurements of the response variable are not independent (here species), which gives rise to a correlated response per species, assumed exchangeable. GEE provides correct marginal or population average models even when the correlation structure is not perfectly specified (Hardin and Hilbe 2003). GEE data were fitted using a function from the 'geepack' package (version 1.2–1; Hojsgaard et al. 2006). The significance of all predictors was tested using the Wald test. Differences between the old and modern records in selected bee species were tested using the non-parametric Mann–Whitney U test. Correlations between all the climate variables and the elevation were expressed in the form of Spearman correlation coefficients. All analyses were performed in the R environment (version 3.5.1; https://www.r-project.org/).

## Results

Bees and wasps preferring steppe biotopes constitute a substantial proportion of the whole fauna of the Czech Republic (253 out of 1297 species, representing 19.5%). Because only 11 species of the steppe specialists are classified as least concern species (LC), the vast majority of species are considered regionally extinct, threatened, or of conservation concern (classified into the categories CR, EN, VU and NT). Species of conservation concern represent more than 96% of the steppe specialists but only 57% of all bee species. Figure 1 shows that the steppe specialists represent the largest proportion of the regionally extinct, critically endangered and endangered species while the proportion decreases in other (lower) categories and is very low among the least concern species. A similar pattern is well-visible also for species preferring open sandy habitats, but it is a bit less notable than for the steppe specialists. Among the eleven least concern species of the steppe specialists, we can find all six species of bees nesting in empty gastropod shells and three cuckoo wasps of the genus *Chrysura* parasitizing in their nests.

There is a clear change in the distribution of the species nesting in empty gastropod shells when comparing their distribution across the two time-slices. *Osmia andrenoides*, a thermophilous bee with its distribution placed in the Mediterranean, is the only species with decreasing populations

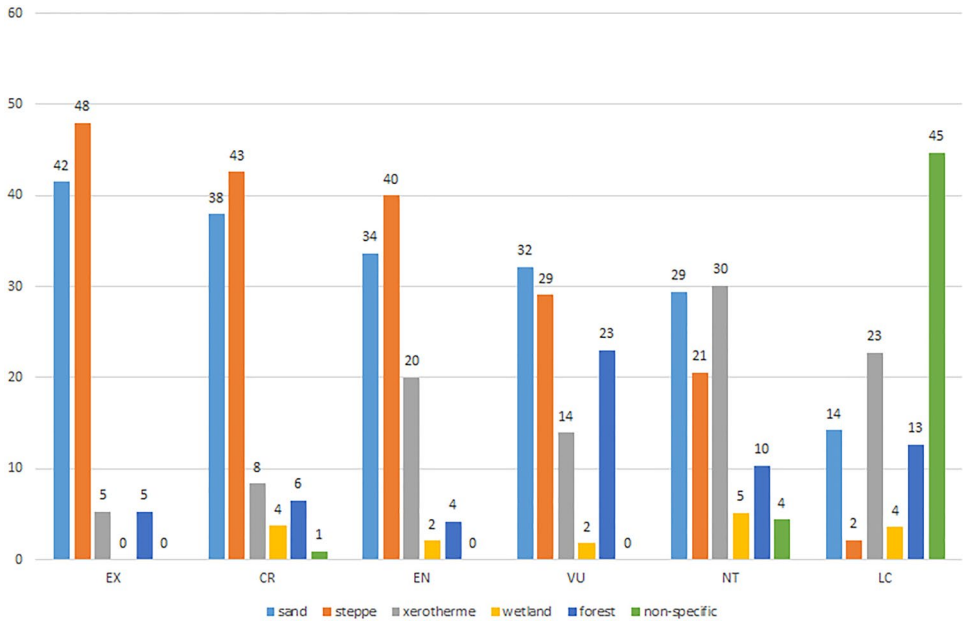
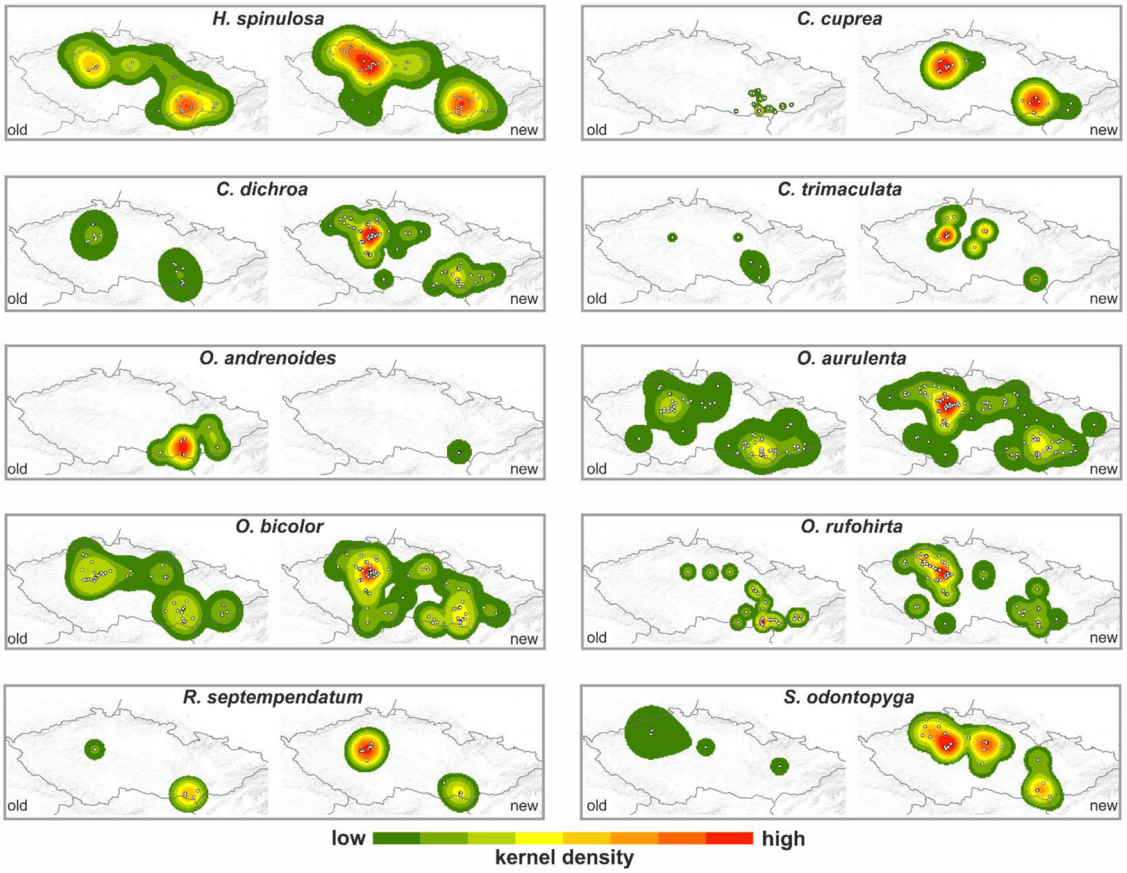



**Fig. 1** Percentage representation of habitat-specialized bees and wasps in red-list categories according to Bogusch and Straka (2017a, b), and Straka and Bogusch (2017a, b). *EX* regionally extinct, *CR* critically endangered, *EN* endangered, *VU* vulnerable, *NT* near threatened, *LC* least concern species. Note that 174 species categorized as data-deficient (DD) and 12 as not-evaluated (NE) were not included in the counts

and also a lower number of localities than in the past. It has its northern distribution limit in the southeast of the Czech Republic and has disappeared from its former localities, except the Pálava Landscape Protected Area, a small limestone mountain range in the southernmost part of the country (Fig. 2). Most of the species, namely *Osmia aurulenta*, *O. bicolor*, *O. spinulosa*, *Rhodanthidium septemdentatum*, and the parasitic *Chrysura dichroa* showed notably similar distributional patterns in both the past and recent distributional maps. Their distribution area has slightly expanded and the number of localities increased. *Osmia rufohirta*, and the parasitic *Chrysura trimaculata* and *Stelis odontopyga* enlarged their areas, expanding to the parts of the country where they have not been previously recorded. The Cuckoo wasp *Chrysura cuprea*, formerly occurring only in the south-eastern part of the country, has recently

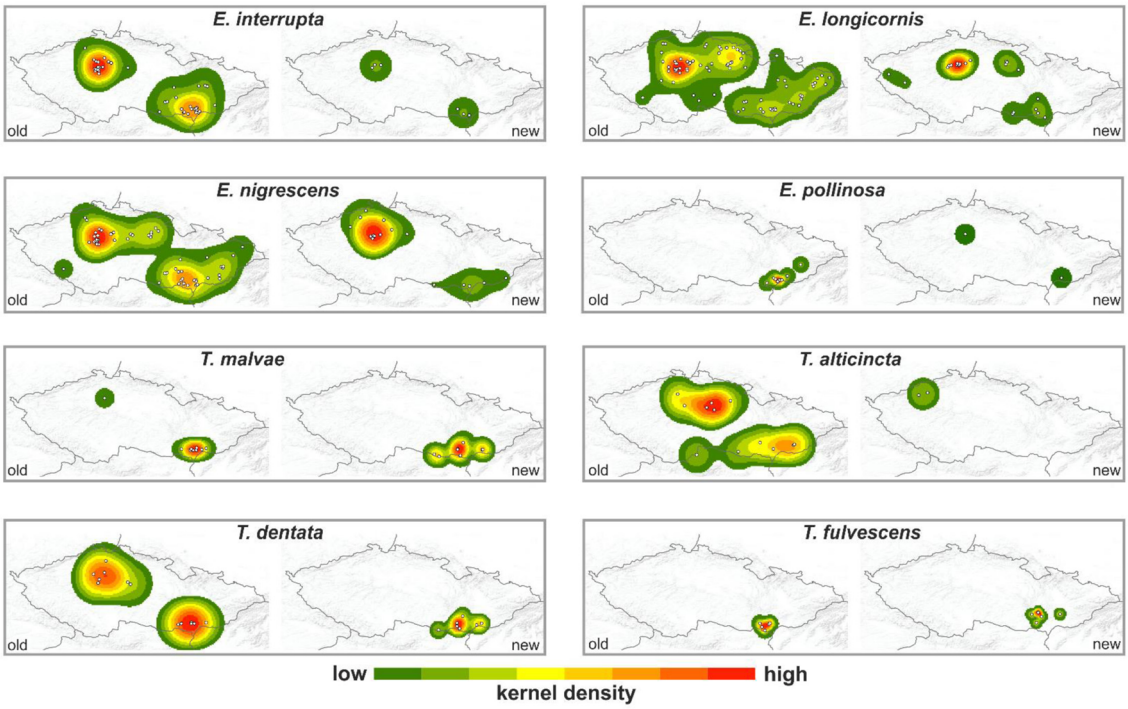



| species | area (km²) | | localities (nr) | | density (localities.km⁻²) | |
|---|---|---|---|---|---|---|
| | old | new | old | new | old | new |
| *Chrysura cuprea* | 4 220 | 17 734 | 23 | 28 | 0.0055 | 0.0016 |
| *Chrysura dichroa* | 9 480 | 34 037 | 17 | 81 | 0.0018 | 0.0024 |
| *Chrysura trimaculata* | 8 012 | 10 236 | 6 | 15 | 0.0007 | 0.0015 |
| *Osmia andrenoides* | 4 111 | 1 | 7 | 3 | 0.0017 | 5.4211 |
| *Osmia aurulenta* | 41 516 | 54 852 | 74 | 133 | 0.0018 | 0.0024 |
| *Osmia bicolor* | 26 469 | 38 415 | 45 | 90 | 0.0017 | 0.0023 |
| *Osmia rufohirta* | 15 184 | 37 589 | 32 | 76 | 0.0021 | 0.0020 |
| *Osmia spinulosa* | 26 568 | 39 629 | 44 | 69 | 0.0017 | 0.0017 |
| *Rhodanthidium septemdentatum* | 2 901 | 5 574 | 8 | 14 | 0.0028 | 0.0025 |
| *Stelis odontopyga* | 1 343 | 14 802 | 4 | 22 | 0.0030 | 0.0015 |

**Fig. 2** The density and clustering of localities for bees nesting in empty gastropod shells and their parasites expressed by the spatial distribution of Kernel density estimation (the higher number of localities per area unit, the higher density). Old (before 1990) and new (after 1990) occurrences are compared for each species separately. Table shows the size of the distribution area in km$^2$ (EOO) in past and present

two hotspots of distribution: one in its former extent of the occurrence and the second far away from this area around the capital Prague and in north-western Bohemia. The extent of the occurrence of all these species (Fig. 2) is larger than it was in the past, while *C. cuprea*, *C. dichroa*, *O. rufohirta* and *S. odontopyga* have enlarged their distribution (EOO) more than twice (*S. odontopyga* more than 10times). The species nesting in empty gastropod shells are virtually the only steppe specialists that are still unendangered, becoming recently even more widespread than in the past.

Ground-nesting bees showed completely opposite results (Fig. 3). Only *Eucera fulvescens* currently occurs in the same region, having a similar distribution, but has recently become rarer than in the past. *Eucera interrupta* occurs in two warm regions of the country but, recently, there have been only several finds, contrary to the numerous records in the past. *Eucera longicornis* and *E. nigrescens* are more common than the others (and thus LC in the red list), but their areas of distribution are much smaller than in the past. *Eucera pollinosa* was newly recorded in Bohemia but nearly disappeared from its former distribution area in the south-east Moravia. *Eucera malvae* and *E. dentata* have become regionally extinct in Bohemia while in Moravia they still survive at several sites. *Eucera alticincta* was twice recorded in northwestern Bohemia while it disappeared from Moravia. All of these species show a substantial decline in their original distribution (see Fig. 3). There are also several species, namely *Eucera caspica*, *E. cineraria*, *E. clypeata*, *E. seminuda*, *E. hungarica*, *E. nana* and *E. salicariae*, which became regionally extinct in the Czech Republic many years ago. However, most of these species have been very restricted also in the past. They occurred in a single area




| species | area (km²) | | localities (nr) | | density (localities.km⁻²) | |
|---|---|---|---|---|---|---|
| | old | new | old | new | old | new |
| *Eucera interrupta* | 21 429 | 2 258 | 41 | 6 | 0.00191 | 0.00266 |
| *Eucera longicornis* | 51 851 | 26 557 | 79 | 24 | 0.00152 | 0.00090 |
| *Eucera nigrescens* | 45 231 | 24 089 | 57 | 19 | 0.00126 | 0.00079 |
| *Eucera pollinosa* | 672 | 1 851 | 9 | 2 | 0.01338 | 0.00108 |
| *Eucera malvae* | 4 027 | 1 918 | 9 | 8 | 0.00223 | 0.00417 |
| *Eucera alticincta* | 23 395 | 25 | 12 | 2 | 0.00051 | 0.07976 |
| *Eucera dentata* | 11 983 | 1 501 | 13 | 7 | 0.00108 | 0.00466 |
| *Eucera fulvescens* | 214 | 841 | 5 | 6 | 0.02337 | 0.00713 |

**Fig. 3** The density and clustering of localities for bees nesting in the ground expressed by the spatial distribution of Kernel density estimation (the higher number of localities per area unit, the higher density). Old (before 1990) and new (from 1990) occurrences are compared for each species separately. Table shows the size of the distribution area in km$^2$ (EOO) in past and present

near the town Čejč in south Moravia, where their localities were changed to an arable land many years ago. For details on these species past distribution see Table 1.

We found important differences between the ground-nesting and the shell-nesting species in the elevation range and mean July temperature of their older and recent records (Fig. 4). While no change was observed for the ground-nesting species (GEE: $p \gg 0.05$), the bees nesting in empty shells and their parasites showed mostly significant shifts of their distribution towards higher elevations (GEE: $X^2_1 = 25.4$, $p \ll 0.001$) and to regions with a lower mean July temperature (GEE: $X^2_1 = 22.6$, $p \ll 0.001$). When testing the changes recorded for individual species, those nesting in shells, i.e. *O. aurulenta*, *O. rufohirta*, *O. spinulosa* and *C. cuprea*, showed significant shifts towards higher elevation and sites of lower mean July temperature. In contrast, ground-nesting *Eucera longicornis* showed the opposite results for July temperature (Fig. 5). The same was true also for another ground-nesting species, *E. interrupta*, though the difference was not significant at $p = 0.05$.

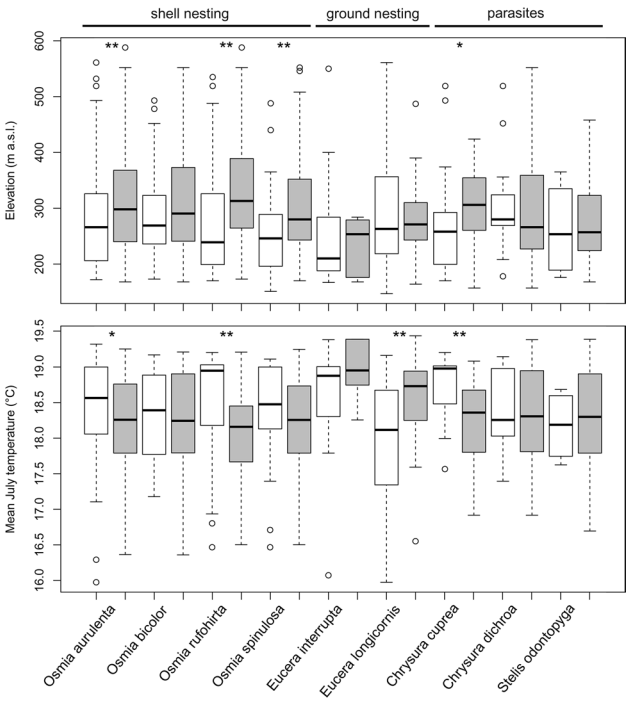



**Fig. 5** Variation in elevation and mean July temperature of old (white) and recent (grey) records of selected bee species. Differences between their old and modern records were tested based on Mann–Whitney U test and significant results are marked by asterisks: *p < 0.5; **p < 0.01. Shell-nesting species: *Osmia aurulenta*, *O. spinulosa*, *O. bicolor* and *O. rufohirta*; ground-nesting species: *Eucera interrupta* and *E. longicornis*. *Chrysura cuprea* is a parasitoid of *O. rufohirta*, *C. dichroa* is a parasitoid of *O. aurulenta* and *Stelis odontopyga* is a cleptoparasite of *O. spinulosa*

## Discussion

Using a comprehensive dataset of historical and recent records for several groups of bees and wasps, we found that species nesting in empty gastropod shells have notably extended their distribution within the Czech Republic. Although we have data only from the Czech Republic, we suppose that the situation is probably similar also in other central European countries. It opens an important question on the causes of this expansion as the species nesting in the ground showed either their distribution contraction or did not show any changes. First, our results suggest that the shell-nesting species were able to colonize new areas at higher elevations and/or with colder summer temperatures, in contrary to the bees nesting in the ground. Furthermore, bees nesting in empty shells also enlarged their EOO but stayed in the regions of their former distribution; in most cases, it seems they formed stronger populations than before (all species shown in Fig. 2). Thus, bees nesting in empty shells positively responded to the current condition in their habitats, forming strong populations therein. It is also interesting that species preferring open, non-overgrown sites (i.e. *O. rufohirta*, *O. spinulosa* and *C. cuprea*) expanded more than species preferring grassy and shrubby slopes (i.e. *O. aurulenta*, *O. bicolor* and *C. dichroa*). This fact can be also connected with the ability of several species to settle in anthropogenic sites such as railway embankments and roadside verges (e.g. Heneberg et al. 2013, 2017; Moroń et al. 2014; Hendrychová and Bogusch 2016) that can provide corridors for their efficient dispersal.

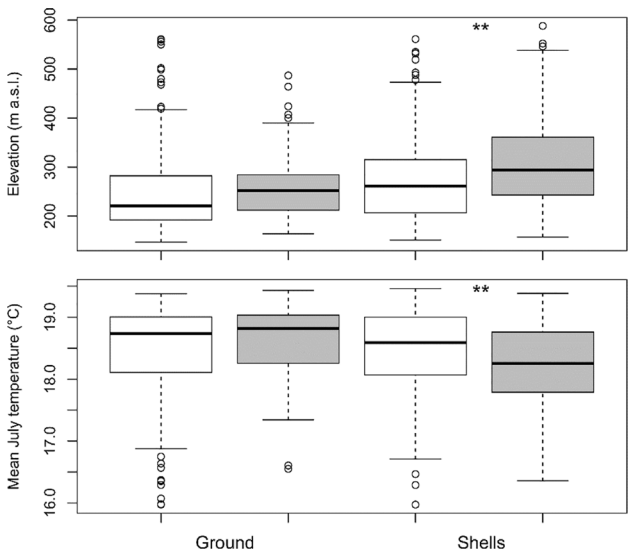



**Fig. 4** Variation in elevation and mean July temperature of old (white) and recent (grey) records of ground-nesting and shell-nesting bees. Significant differences found in shell-nesting species, tested based on GEE: $X^2_1 = 25.4$, $p \ll 0.001$ (elevation) and $X^2_1 = 22.6$, $p \ll 0.001$ (temperature), are marked by asterisks (**)

The increase in the EOO for shell nesting species, but the decline of those nesting in the ground, are highly connected with the general landscape changes. The inspection of historical and recent orthophoto maps supports this assumption. Some of the main localities have been changed already during the nineteenth century, but the bees occurrence can be tracked until the second half of the twentieth century. It seems though that the species loss was caused mainly by the intensification of agriculture than by the change of their habitats. Some sites were changed into fields (the landscape around the towns of Čejč and Kobylí, see Kiliánová et al. 2009) and the others were afforested (the sandy area around the town of Hodonín and Bzenec, see Jedlička et al. 2019). The last remnants of these steppes were spontaneously overgrown by shrubs and trees. Botanical data show only 5–10% the steppe area was covered by trees and shrubs, in contrast to 30–40% today (Chytrý et al. 2020), although these sites are under official conservation for many years. The current deterioration of steppe grasslands was found to explain why there is a higher species extinction at these habitats in lowland than in sub-mountain regions (Divíšek et al. 2020). If remained open, these sites were at least invaded and overgrown by non-native neophyte grasses (Wagner et al. 2012; Pokorný et al. 2015) and other plants: the steppes in the Pálava PLA recently suffer from spreading of *Isatis tinctoria* (P. Bogusch, unpublished observations). These changes, which are associated with the total absence of bare ground patches, with a tough ground unsuitable for the nesting and lower diversity of flowering plants, resulted in a substantial reduction or extinction of the populations of species nesting in the ground. It was reported that the reduction of these species occurrence is linked with the lack of nesting places and smaller or different availability of flowering plants as food for the adults and their brood (Westrich 1996; Kosior et al. 2007; Nieto et al. 2014; Přidal and Veselý 2011; Rasmont et al. 2015).

On the contrary, high productive shrubby vegetation overgrowing steppe remnants provide suitable conditions for many large-body snails which enlarged their EOO and population densities at these localities (Peltanová et al. 2012; Juřičková et al. 2014). Recent surveys showed that large-body snail species (e.g. *Helix pomatia* Linnaeus, 1758, *Caucasotachea vindobonensis* (Férussac, 1821) and *Fruticicola fruticum* (Müller, 1774)) became dominant at many steppe sites that were more overgrown by shrubs, while early-succession stages at anthropogenic sites, such as spoil heaps, former military areas or roadside verges, showed dominance of middle-size species, usually *Xerolenta obvia* (Menke, 1828) and *Monacha cartusiana* (Müller, 1774). These habitats were found to have a higher density of empty shells than the steppe reserves (Bogusch et al. 2019). If the presence and abundance of nesting sites, i.e. empty shells, is the most limiting factor for the shell-nesting bees, then the increase in available snail shells is an expected condition for the spread of species nesting in shells.

The spread of bees nesting in empty shells could also be connected with the spread of some snail species, even though there are only a few species known to be expanding their ranges in central Europe over the last 100 years. Mediterranean *Monacha cartusiana* was previously known only from the southeast part of the Czech Republic, while over the last 20 years it has colonized most of lowland and warm areas across the whole country (Peltanová et al. 2012). Currently, this species is typical for xerothermic post-industrial sites and the sites highly influenced by humans such as spoil heaps, roadside verges and railway embankments, while it has a scattered occurrence in the agricultural landscape and steppe formations (Peltanová et al. 2012). As the species develops strong populations and has quite a short lifespan (Staikou and Lazaridou-Dimitriadou 1990; Lesicki and Koralewska-Batura 2007), the localities are full of empty shells suitable for bee nesting. Several smaller bee species settle in the shells of *M. cartusiana* as confirmed by Bogusch et al. (2019) who reared *O. rufohirta*, *O. spinulosa* and its cuckoo bee *S. odontopyga* from shells of this snail species. Müller et al. (2018) also observed *O. rufohirta* nesting in shells of this snail.

An important conservation question is whether we can do something to make the steppe formations again suitable for both the ground-nesting and the shell-nesting species. A sharp decline of many species is linked with landscape changes that happened approximately over the last 100 years, being connected with the land change by agriculture and forestry (Jongman 2002; Walz 2008; Skaloš et al. 2011; Skokanová et al. 2012). Sandy sites or areas that were impossible to use for planting crops, have been afforested (Balej 2012; Lorencová et al. 2013). Even many protected sites have been invaded and overgrown by invasive plants because the nature conservation efforts were not sufficient, or no management interventions have been applied (Tickle 2000; Oszlányi et al. 2004). As a result, remnants of steppe formations have changed into shrubby slopes or meadow formations (Lorencová et al. 2013). Recently, the implementation of appropriate conservation measures has resulted in some improvement in this situation. Several species recently have started to increase their occurrence the central European landscape because many of them were able to utilize artificial, usually anthropogenic, sites of early succession such as sandpits (Heneberg et al. 2013), spoil heaps (Tropek et al. 2013; Hendrychová and Bogusch 2016), roadside verges and railway embankments (Moroń et al. 2014; Heneberg et al. 2017). Although most of these sites have scattered distribution with long distances between them, populations of many invading or returning species can spread into new sites and many of these species become quite common and numerous in some regions (e.g. *Bembix tarsata* Latreille, 1809, *Sphex*

*funerarius* Gussakovskij, 1934 and some others, see Srba and Tyrner 2003; Hendrychová and Bogusch 2016). The recovery observed in several species might also be linked with climate change in recent decades, however, clear evidence for this is missing. Therefore it is possible that some steppe habitats, especially those with suitable management practices, will become shortly also suitable for species nesting in the ground. However, to fully restore the habitats to their conditions and extent documented about 100 years ago is a long-term process requiring the regular implementation of new management practices.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Research involved in human and animals participants** No human participants and animals were used within this research.

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**5.4 Bogusch P., Hlaváčková L., Rodriguez-Gasol N., Heneberg P. 2020b: Near-natural habitats near almond orchards with presence of empty gastropod shells are important for solitary shell-nesting bees and wasps. *Agriculture, Ecosystems and Environment* 299: 106949. https://doi.org/10.1016/j.agee.2020.106949.**

Výzkumy probíhaly na přírodě blízkých i ruderálních stanovištích sousedících s mandloňovými sady nedaleko města Lleida v severovýchodním Španělsku. Sadů bylo celkem 12 a byly rozděleny do tří kategorií na základě jejich umístění a zavlažování. Naším cílem bylo zjistit, zda je výskyt druhů ulitových včel limitován přítomností prázdných ulit na přírodních stanovištích a jestli trendy v početnosti včel v prázdných ulitách korespondují s výsledky našich předchozích výzkumů provedených ve střední Evropě. Dále byly zkoumány parametry ovlivňující složení ulitových druhů včel na přírodě blízkých stanovištích sousedících s mandloňovými sady. Dílčím cílem bylo objasnit, jak dané druhy hnízdí.

Byly použity dvě metody: sběr přirozeně se vyskytujících prázdných ulit z povrchu země a experimentální nabídka označených prázdných ulit na lokalitu.

Sběr ulit proběhl v březnu 2019, přičemž z každé lokality jsme se snažili sesbírat alespoň 400 kusů. Materiál byl následně rozebrán v laboratoři a získaný hmyz fixován v ethanolu a určen.

Barevně označené ulity patřily čtyřem velkým nebo středně velkým druhům plžů běžných druhů (*Cornu aspersum, Eobania vermiculata, Cernuella virgata* a *Pomatias elegans*) a na lokalitu jsme je umístili začátkem dubna 2018, krátce před hnízděním námi studovaných včel. Ulity byly pokládány na zem v sadách po čtyřech, přičemž v každé této skupině byly zastoupeny schránky všech čtyř druhů plžů (od každého druhu jedna). Na každou lokalitu jsme těchto sad umístili 40, dohromady tedy připadalo na jednu lokalitu 160 ulit. Ve většině případů byly sady ulit umísťovány v linii ve vzdálenosti cca 1–2 metry od sebe a označeny barevným plastovým brčkem pro budoucí snazší dohledání. V terénu byly ponechány do konce září, tedy do závěru hnízdní sezóny. Materiál byl následně převezen do laboratoře, kde byl rozebrán, dospělí blanokřídlí byli konzervováni v 96 % ethanolu a identifikováni do druhu (či alespoň rodu). Larvy a kukly byly přendány do malých mikrozkumavek, uzavřeny zátkou z papírové utěrky a ponechány k vylíhnutí. Vylíhlí dospělci byli opět konzervováni v ethanolu a určeni.

V přirozeně se vyskytujících ulitách jsme objevili 23 druhů, přičemž u 15 z nich se jednalo o hnízdící druhy, zbylých 8 bylo parazitických (parazitoidi i kleptoparaziti). Většina pozorovaných druhů patřila do čeledi Megachilidae (12 hnízdících a 1 kukaččí včela) – nejčastěji zachycenými druhy byly *Osmia ferruginea* a *Rhodanthidium sticticum*. Mimo

blanokřídlých jsme zaznamenali i jejich parazity z jiných řádů – dlouhososku *Anthrax aethiops* (Diptera) a pestrokrovečníka *Trichodes apiarius* (Coleoptera).

Barevně označené ulity byly zahnízděné jen velmi zřídka. Nalezli jsme zde 10 druhů ze 4 čeledí žahadlových blanokřídlých, z toho 8 druhů hnízdících (nejpočetněji byl zastoupen druh *O. aurulenta*) a 2 parazitické (*S. quinquepunctata, C. rufiventris*). Jediným druhem zaznamenaným v nastražených ulitách, který zároveň chyběl v ulitách přirozeně se vyskytujících, byl druh *Protosmia exenterata* (Pérez, 1896).

Výzkumem jsme prokázali, že vysoký podíl prázdných ulit na lokalitách je opravdu nezbytný pro přítomnost v ulitách hnízdících druhů včel. Dalším nezbytným předpokladem je pak přítomnost kvetoucích rostlin jakožto zdroje potravy pro dospělce i larvy včel. Oba zdroje, hnízdní i potravní, jsou dostupné především na přírodě blízkých stanovištích, které tak mohou sloužit jako ohniska biologické rozmanitosti včel. Přítomnost mikrobiotopů přírodního charakteru v blízkosti sadů je zároveň velmi důležitá i pro pěstované plodiny v sadech.

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# Near-natural habitats near almond orchards with presence of empty gastropod shells are important for solitary shell-nesting bees and wasps

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ABSTRACT

Solitary bees serve as important native pollinators of wild plants and crops. Several species of bees, which form strong populations in open habitats of European landscapes, nest in empty snail shells. In the present study, we focused on shell-nesting bees in near-natural habitats neighbouring almond orchards near the town Lleida in north-east Spain. We performed a series of manipulative experiments, which aimed to elucidate the importance of empty shells of four common large- and medium-sized gastropod species for nesting bees in near-natural and ruderal habitats surrounding the almond orchards. We also matched these data with findings of bees in naturally occurring shells at each examined site. All study sites consisted of habitats with naturally present shells and evidence of the natural presence of the study species of bees. In total, we recorded 15 nesting bee and wasp species and eight species of hymenopteran parasites in collected shells, one of which, the spider wasp *Priocnemis propinqua*, was recorded nesting in empty gastropod shells for the first time. In total, the deployed shells hosted eight nesting species and two species of parasitic Hymenoptera. The prevalence of nests in experimentally deployed shells was low, with only a single nest present near irrigated orchards. The abundance of nests was also low at sites where the naturally present shells were highly abundant. Combined, available evidence suggests the importance of near-natural habitats for the presence of shell-nesting bees and wasps in South-European landscapes. The abundance of shell nesting bees and wasps does not increase proportionally with the increase in empty snail shell abundance and other factors should be considered limiting at such sites.

## 1. Introduction

Solitary bees, which often form dense and large populations, are increasingly recognized as important native pollinators, even in agricultural landscapes (Potts et al., 2010). Several of them, such as the leafcutter bee *Megachile pacifica* (Panzer, 1798) (syn. *Megachile rotundata* (Fabricius, 1784)) or mason bees *Osmia cornuta* (Latreille, 1805) and *Osmia tricornis* Latreille, 1811 are frequently kept in trap-nests in the vicinity of alfalfa fields or fruit orchards. Although they were previously overlooked, they play a key role in pollination of various crops (Bosch and Blas, 1994; Pitts-Singer and Cane, 2011; Alomar et al., 2018; Herrmann et al., 2018). Several species of the family Megachilidae also form dense and large populations in warm and dry localities while the most numerous and widespread species usually nest in empty shells of larger gastropods. These species of bees are usually polylectic or broadly oligolectic (for review of the ecology of European species, see Müller et al., 2018) and can be of high importance for pollination of various crops, including fruit trees, strawberries, alfalfa, or clover. Because open, inclined, south-exposed habitats with shrubs are usually full of empty shells of large- or medium-sized gastropods, shell nesting bees are abundant at these sites and can serve as important native pollinators of crops planted at or around their nest sites.

Bees are the most important pollinators of fruit trees, and the majority of bee species use the fruit trees as an important or preferred source of nectar and pollen but make their nests in small patches of near-natural habitats around the orchards (Bosch and Kemp, 2002; Henselek et al., 2018). The combination of these habitats is important not only for the bees but for many other species and groups of plants and animals (Scott-Dupree and Winston, 1987; Kremen et al., 2002; Carvalheiro et al., 2010). Almond trees are pollinated by honeybees (Danka et al., 2006; Klein et al., 2012), bumblebees (Dag et al., 2006), and mason bees (Bosch et al., 2000), with the importance of wild (native) pollinators only recognized in recent. Available evidence

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suggests that the occurrence of wild bees in near-natural patches near the orchards is highly beneficial for crop production years (Klein et al., 2012).

We aimed to address the composition of bee species nesting in empty gastropod shells in near-natural sites neighbouring almond orchards in north-eastern Spain. We used two methods: 1) collecting naturally occurring empty shells from the ground surface, and 2) experimental deployment of empty shells of four large or medium-sized common species (*Cornu aspersum* (O. F. Müller, 1774), *Eobania vermiculata* (O. F. Müller, 1774), *Cernuella virgata* (Da Costa, 1778) and *Pomatias elegans* (O. F. Müller, 1774)). We aimed to find out if the bee species are limited by the presence of shells in natural habitats and if the trends in abundance of bees in empty shells correspond with the results of our previous surveys done in central Europe (Bogusch et al., 2019; Heneberg et al., 2020). In addition, the habitat parameters affecting the composition of bees nesting in empty shells in near-natural habitats neighbouring almond orchards were also investigated.

## 2. Materials and methods

The study was done in the vicinity of Lleida in north-eastern Spain in near-natural or ruderal habitats neighbouring with 12 almond orchards (Table 1), which were categorized into three groups according to location and irrigation. Four orchards were located in the river floodplain and were not irrigated. Four other orchards were located in a semi-humid area in the river floodplain and were irrigated. The remaining four orchards were located in a dry hilly region several kilometres away from the river floodplain. The localities were small in area (from 690 to 30,993 square meters) and were 0–500 m far from the orchard. At the beginning of April 2018, we collected empty shells of adults of four common South-European species of terrestrial gastropods – *C. aspersum* (syn. *Helix aspersa*), *E. vermiculata*, *C. virgata* and *P. elegans*. These species differ in size (*C. aspersum* is the largest while *P. elegans* the smallest) and in the overall shape of the shell. The shell of adult *C. aspersum* is round and reaches a width between 30 – 40 mm, *E. vermiculata* is slightly flattened and reaches a width between 24 – 32 mm, *C. virgata* is flattened and reaches a width between 15 – 23 mm, and *P. elegans* is elongated and reaches a width 9 – 12 mm (Welter-Schultes, 2012). All species are abundant in open xerothermic habitats across Southern Europe, and empty shells of these species are preferentially utilized by solitary shell-nesting bees (see also Müller et al., 2018 for more detailed information on shells used by shell-nesting bees). We cleaned the shells, removed their inner contents, washed them in water, dried them, and sprayed all of them with red colour acrylic lacquer. The red colour was used to make the shells more easily visible when collecting them but did not disturb the bees because the red colour is not conspicuous for the bees (see Chittka and Waser, 1997; Briscoe and Chittka, 2001). Coloured shells were left in the air-conditioned room for at least 48 h before deploying in the localities. We deployed 40 shells of each species at every locality in a line of groups of four (one of each species) marked with a plastic drinking straw to further support the visibility of deployed shells at the locality (see Fig. 1). We deployed all the shells in early April - shortly before the first shell-nesting bees begin to search for potential nesting microhabitats. We retained the shells at the localities until the end of the nesting season (late September) when we collected all of them and extracted the inner contents of the shells within several hours or days after the collection. We preserved all adult bees and other Hymenoptera in 96 % ethanol and identified to the species or genus level. We transferred all the larvae and pupae into plastic 1.5 ml micro-tubes, closed them with small plug of paper towel and incubated them under laboratory conditions (temperature ~21 °C, humidity 45%–50%). During the winter months (November through January), we moved the larvae into the climate box at 8 °C and 60 % humidity in order to allow hibernation. In

**Fig. 1.** Photo of a group of experimentally deployed shells of four species marked with a drinking straw.

**Table 1**
List of the localities with their characters. The codes are the normally used numbers of orchards in IRTA, Lleida.

| Number | GPS orchard [°N, °E] | GPS near-natural site [°N, °E] | Area of near-natural site (m2) | Number of shells | Natural character | Water management |
|---|---|---|---|---|---|---|
| W1 | 41.527240, 0.830040 | 41.525840, 0.829120 | 4 666 | Medium | Ruderal | Wet |
| W2 | 41.545249, 0.868950 | 41.544897, 0.869304 | 3 051 | High | Ruderal | Wet |
| W3 | 41.524514, 0.850238 | 41.524264, 0.850429 | 690 | Low | Ruderal | Wet |
| W4 | 41.514432, 0.777764 | 41.513644, 0.777150 | 8 535 | High | Natural | Wet |
| I1 | 41.612270, 0.717278 | 41.611166, 0.717175 | 6 330 | Medium | Natural | Irrigated |
| I2 | 41.602552, 0.779964 | 41.603300, 0.780262 | 1 055 | High | Ruderal | Irrigated |
| I3 | 41.612180, 0.929008 | 41.612494, 0.929254 | 5 464 | Low | Ruderal | Irrigated |
| I4 | 41.609812, 0.850119 | 41.609420, 0.850906 | 3 022 | Low | Natural | Irrigated |
| D1 | 41.515268, 0.660402 | 41.515791, 0.661132 | 30 993 | Medium | Natural | Dry |
| D2 | 41.497948, 0.685318 | 41.497582, 0.683386 | 4 865 | Medium | Natural | Dry |
| D3 | 41.485608, 0.756119 | 41.485716, 0.755620 | 4 143 | Low | Natural | Dry |
| D4 | 41.519444, 0.942408 | 41.519978, 0.942520 | 16 581 | Medium | Natural | Dry |

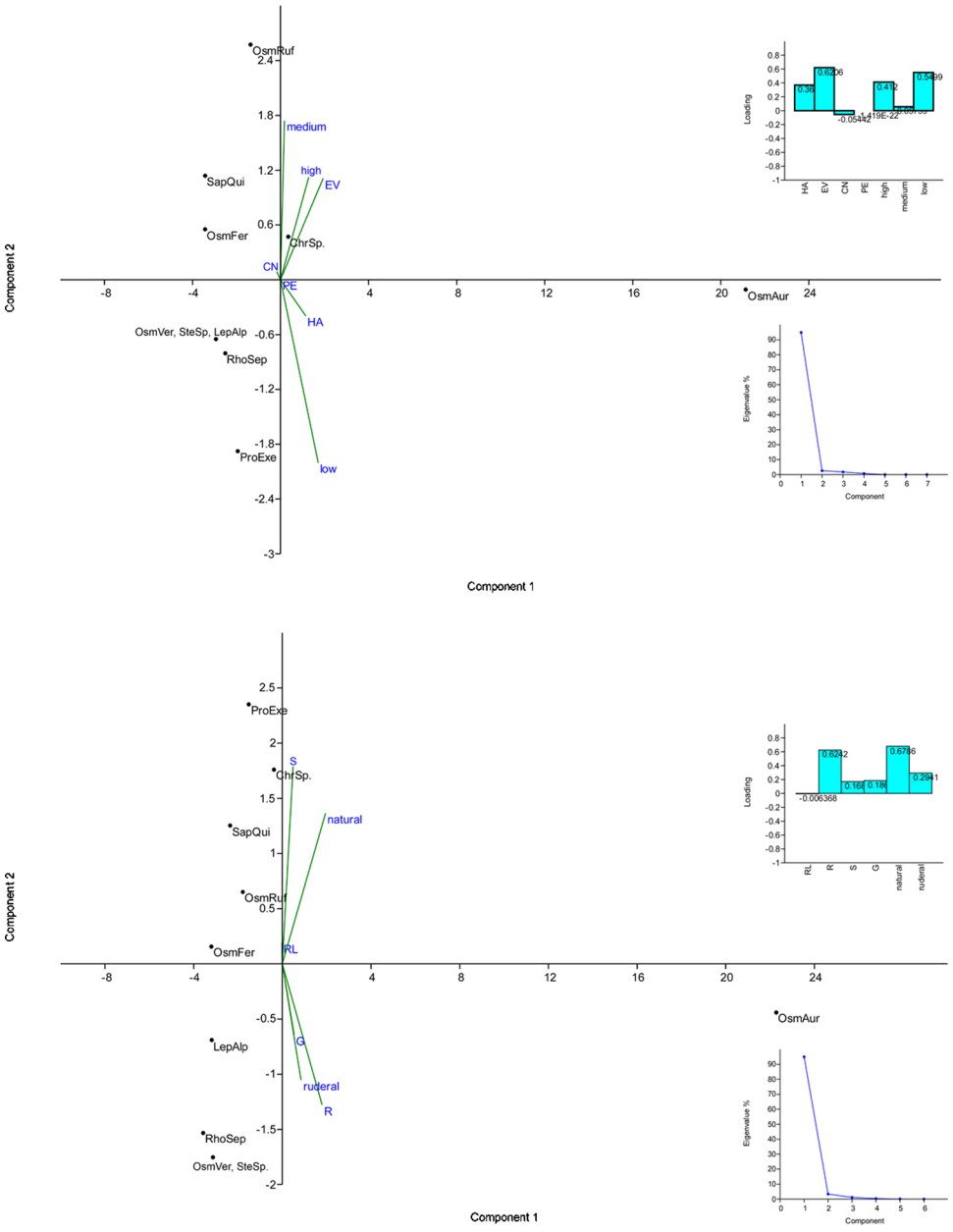


**Fig. 2.** (A) Results of the detrended correspondence analysis of the numbers of nests of aculeate species, which utilized empty shells with (A) shell species (HA - *Cornu aspersum*, EV - *Eobania vermiculata*, CN - *Cernuella virgata*, PE - *Pomatias elegans*) and the number of native shells at the habitat (low, medium, high). Variance explained by eigenvalues: PC1 = 94.89 %, PC2 = 2.60 %. (B) Results of the canonical correspondence analysis of the numbers of nests of aculeate species, which utilized empty shells and the orchard types (R - wet not-irrigated, RL - wet and irrigated, S - dry, G - near-natural habitats in and around Garraf National Park) and habitat type (natural vs. ruderal). Variance explained by eigenvalues: PC1 = 94.96 %, PC2 = 3.36 %.

January, we moved the larvae back to laboratory conditions and allowed the bees to eclose. After eclosion, we preserved the bees and other reared insects in 96 % ethanol and identified them to species.

In the first half of March 2019, we collected around 400 empty terrestrial snail shells per sampling site (mean 543; range 75–647) that were visible on the surface at the same localities where the shells were deployed during the previous summer season. The spectrum of collected shells fully reflected the spectrum of larger shells that were both suitable for nesting and available at the sites. Sampling could potentially be biased against bee species that only use shells present deep in the leaf litter, under the stones or in soil burrows. We opened all the shells similarly to the coloured experimentally deployed shells. We fixed the obtained and/or reared insects in 96 % ethanol.

To compare the localities, we estimated the number of empty gastropod shells per square meter by counting empty shells that were present at five randomly selected places within each examined locality. We counted only the shells of medium-sized and large species. We divided the localities into the following three categories depending on the number of empty shells per square meter, low (< 20 per $m^2$), medium (20–50 per $m^2$) and high (> 50 per $m^2$). We also classified the localities according the vegetation type as near-natural (with mostly native vegetation and less than 20 % of ruderal, non-native or expansive plants) and ruderal localities (all other sites). The plant cover of near-natural sites was herbal or shrubby (height to 0.5 m) vegetation with *Rosmarinus*, *Ulex*, *Lithodora* and several Brassicaceae species being the dominant plants, while *Rubus*, *Capparis* and Asteraceae were most numerous at ruderal sites.

We recorded the number of individuals and species of all aculeate Hymenoptera and their parasites that were present in the shells. To estimate their species richness, we calculated the Chao-1 estimator, corrected for unseen species. We also computed the rarefaction curves based on the log gamma function for computing combinatorial terms (Krebs, 1989). The rarefaction procedure consists of selecting a specified number of samples that is equal to or less than the number of samples in the smallest sample, and then randomly discarding reads from larger samples until the number of remaining samples is equal to this threshold (Willis, 2019). To compare species richness of the analysed datasets, we calculated the Sørensen similarity index. We calculated the basic diversity indices for

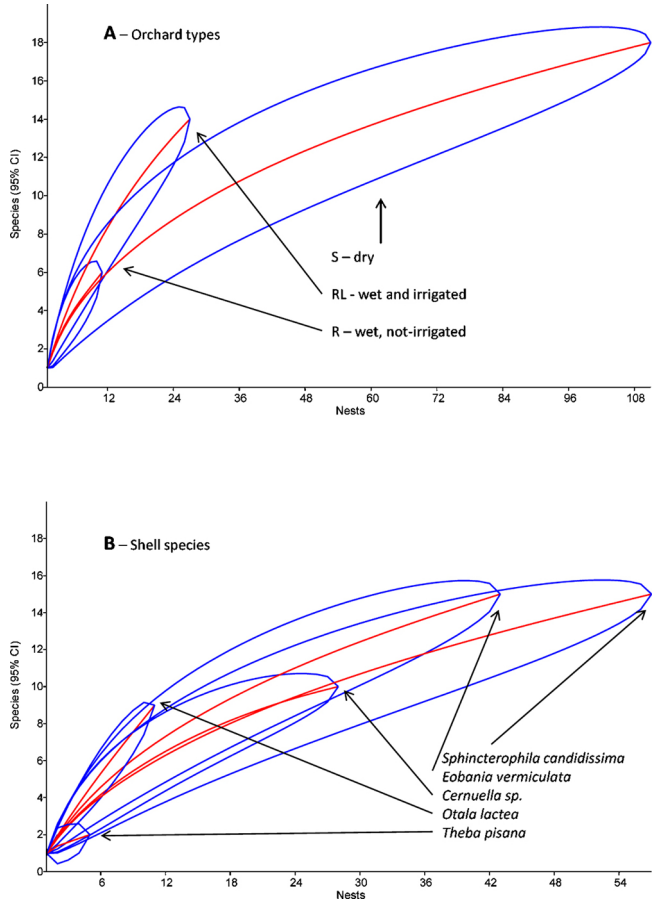


**Fig. 3.** Rarefaction curves of the numbers of nests of aculeate species, which utilized empty shells, and which were stratified according to (A) the orchard types and (B) the shell species used. Orchard types: (R - wet and not-irrigated, RL - wet and irrigated, S – dry). Analysed shell species: *Sphincterophila candidissima*, *Eobania vermiculata*, *Cernuella* sp., *Otala lactea*, and *Theba pisana*. Data are shown for the naturally present shells. Numbers on the X-axis indicate the number of nests of bees or wasps when the nest was counted once for each bee or wasp species recorded. The rarefaction curves are accompanied with the visualization of 95 % confidence intervals.

each dataset. These data included species dominance (expressed as 1 – Simpson index, where 1 indicates the complete domination of a single species, and 0 indicates the equal representation of all taxa), the equitability (the evenness measure, in which the Shannon index is divided by a logarithm of the number of taxa), Fisher's alpha (diversity measure), and the Berger-Parker dominance index (the number of individuals in the dominant species relative to the total number of individuals). To compare the indices, we employed bootstrapping (at n = 1000) and permutation analysis. We compared the species richness of the analysed datasets using the Sørensen similarity index and assessed the differences in host species-specific diversities between the analysed time points using Shannon diversity *t*-test. We used detrended correspondence analysis to assess the contribution of analysed factors. We used $\chi^2$ test with Yates correction to analyse the differences in species in habitats with various numbers of empty shells. We performed all the calculations in SigmaPlot 12.0, EstimateS 9.1.0 and PAST 2.14. Data are shown as mean ± SD unless stated otherwise.

## 3. Results

### 3.1. Naturally present shells

We reared 345 bee and wasp individuals from a total of 150 nests, i.e. 2.4 % of the 6,267 examined shells were occupied by nesting bees or wasps. The reared individuals belonged to 23 species, of which 15 were nesting species and eight were their parasites (parasitoids and nest cleptoparasites). A detailed list of species retrieved from both samples from each examined site is provided in Table S1. Most of the species recorded belonged to the family Megachilidae (12 nesting and one cuckoo bee), with *Osmia ferruginea* Latreille, 1811 and *Rhodanthidium sticticum* (Fabricius, 1787) representing the most abundant species. We also recorded a parasitic fly *Anthrax aethiops* (Fabricius, 1781) (Diptera: Anthracidae) and a beetle *Trichodes apiarius* (Linnaeus, 1758) (Coleoptera: Cleridae) in Hymenoptera nests; both species are common parasites of shell-nesting bees and they were also included in the analyses. The species were disproportionately distributed among the three habitat types. Most nests were recorded in dry habitats (287 individuals of 15 species in 106 nests). Much lower number of nests were in irrigated habitats (16 individuals of 7 species in 15 nests) and in wet habitats in river floodplain (41 individuals of 13 species in 29 nests) ($\chi^2$ test $p < 0.001$ for nests and individuals, respectively, when compared to random distribution; however, $p > 0.05$ for the number of species; see Fig. 2A for detrended correspondence analysis).

### 3.2. Experimentally deployed shells

From the marked shells that were deployed throughout the nesting season, we reared 41 individuals of 10 species of aculeate Hymenoptera that we identified to the species or genus level. A detailed list of species retrieved from both samples from each examined site is provided in Table S2. Only 17 of 1,920 shells (0.89 %) were occupied by nesting Hymenoptera. Of the recorded ten species of four families of aculeate Hymenoptera, two are parasitic (*Sapyga quinquepunctata* (Fabricius, 1781) (Sapygidae) and *Chrysura rufiventris* (Dahlbom, 1854) (Chrysididae)). This means that eight species were nesting inside the deployed shells. *Osmia aurulenta* (Panzer, 1799) (Megachilidae) was the most numerous nesting species with eleven nests containing 26 individuals while the other species were much less abundant – *Osmia rufohirta* Latreille, 1811 (Megachilidae) and *C. rufiventris* both with two nests and three individuals, and the other species with only one nest occupied. *Protosmia exenterata* (Pérez, 1896) (Megachilidae) was the only species recorded in deployed shells but not recorded in collected shells.

### 3.3. Preference for habitat type

We found more species at near-natural sites with native vegetation - 21 species were recorded in collected shells in near-natural sites in contrary to only five species recorded in ruderal sites. Rarefaction (Fig. 3A) revealed that this difference was associated with lower occupancy of shells but not lower species richness in other habitat types. There were similar differences in numbers of nests (135 vs 15) and total individuals found (330 vs 35) ($\chi^2$ test $p < 0.001$ for nests, individuals and species, respectively, when compared to random distribution; see Fig. 2A for detrended correspondence analysis). The difference in marked shells was not as prominent since six species were recorded in near-natural sites and four species originated from ruderal sites; the number of nests was 13 vs 6 and the number of individuals was 24 vs 10. The Chao-1 estimator, which was calculated based on the number of nests found, suggested that the examined dataset was incomplete and it indicated the presence of 12 bee and wasp species in naturally present shells in wet, not irrigated orchards, 21 species in wet irrigated orchards and 27 species in dry orchards in the study area (Table 2). The studied assemblages displayed significant differences in dominance ($p < 0.05$ by bootstrapping; Table 2), with the highest values found in wet, non-irrigated orchards, followed by dry orchards and with lowest dominance in wet irrigated orchards. The equitability and Fisher's alpha did not differ significantly among the three types of analysed orchards ($p > 0.05$ by bootstrapping; Table 2). The bee and wasp

**Table 2**
Species composition, species richness and diversity of bees and wasps nesting in empty, naturally present snail shells in analysed orchard types (R – wet, not-irrigated, RL - wet and irrigated, S – dry). Data are provided separately for naturally present shells and experimentally deployed shells. The selected species diversity indices are indicated.

| Orchard type Index | R | RL | S | Total | Bootstrapping *p* for totals (natural vs deployed) |
|---|---|---|---|---|---|
| NATURALLY PRESENT SHELLS: | | | | | |
| Number of bee and wasp nests found | 11 | 27 | 111 | 149 | 0.000 |
| Number of bee and wasp species found | 6 | 14 | 18 | 23 | 0.007 |
| Chao-1 ± SD | 11.5 ± 6.4 | 20.7 ± 6.4 | 27.2 ± 8.8 | 30.0 ± 6.6 | |
| Dominance | 0.240 | 0.119 | 0.201 | 0.170 | 0.006 |
| Equitability | 0.890 | 0.907 | 0.715 | 0.742 | 0.707 |
| Fisher's alpha | 5.400 | 11.710 | 6.088 | 7.603 | 0.076 |
| Berger-Parker dominance index | 0.360 | 0.259 | 0.360 | 0.342 | 0.009 |
| Shannon *t*-test of totals ($t$; $d_f$; $p$): | | | | | |
| - Naturally present vs. experimentally deployed | | | | | 3.6; 53.0; 0.0006 |
| Sørensen similarity index: | | | | | |
| - Naturally present, RL vs indicated cohort | 0.50 | | | | |
| - Naturally present, S vs indicated cohort | 0.50 | 0.56 | | | |
| - Exp. deployed, R vs indicated cohort | 0.40 | 0.44 | 0.27 | | |
| - Exp. deployed, RL vs indicated cohort | 0.00 | 0.00 | 0.11 | | |
| - Exp. deployed, S vs indicated cohort | 0.50 | 0.30 | 0.50 | | |
| - Naturally present, total vs indicated cohort | 0.41 | 0.76 | 0.88 | | |
| - Exp. deployed, total vs indicated cohort | 0.57 | 0.46 | 0.54 | 0.52 | |
| EXPERIMENTALLY DEPLOYED SHELLS: | | | | | |
| Number of bee and wasp nests found | 19 | 2 | 13 | 34 | |
| Number of bee and wasp species found | 4 | 1 | 7 | 9 | |
| Chao-1 ± SD | 6.8 ± 4.1 | 1.0 ± 0.3 | 6.3 ± 0.9 | 9.0 ± 1.8 | |
| Dominance | 0.718 | 1.000 | 0.172 | 0.344 | |
| Equitability | 0.440 | 0.000 | 0.948 | 0.701 | |
| Fisher's alpha | 1.546 | 0.796 | 6.182 | 3.996 | |
| Berger-Parker dominance index | 0.842 | 1.000 | 0.231 | 0.559 | |
| Sørensen similarity index: | | | | | |
| - Exp. deployed, RL vs indicated cohort | 0.00 | | | | |
| - Exp. deployed, S vs indicated cohort | 0.40 | 0.29 | | | |
| - Naturally present, total vs indicated cohort | 0.30 | 0.08 | 0.41 | | |
| - Exp. deployed, total vs indicated cohort | 0.67 | 0.22 | 0.86 | | |

assemblages in wet, non-irrigated orchards and in dry orchards both had significantly higher Berger-Parker dominance index compared to wet irrigated orchards ($p < 0.05$ by bootstrapping; Table 2). The Shannon *t*-test indicated that the diversity of bees and wasps in the three analysed orchard types differed from one another. The Sørensen similarity indices indicated that there was a low similarity among the bee and wasp assemblages in the three analysed orchards (0.50 – 0.56). Interestingly, even lower values for the Sørensen similarity index were reached when comparing the bee and wasp assemblages in naturally present and experimentally deployed shells within the same orchard types or with any other examined orchard types (Table 2).

### 3.4. Snail shell preferences by nesting species – naturally present shells

Altogether, we collected 6,276 shells of nine species of terrestrial gastropods. Rarefaction (Fig. 3B) revealed that the bee and wasp species richness was roughly similar in shells of different snail species despite strong differences in the occupancy rates. The shells of *Otala lactea* (O. F. Müller, 1774) were associated with the highest (but incompletely sampled) species richness and the shells of *Theba pisana* (O. F. Müller, 1774) were associated with the lowest species richness and occupancy rate among the five snail species that were analysed in Fig. 3B. In agreement with the rarefaction analysis, the Chao-1 estimator, which was calculated based on the number of nests found, indicated the highest species diversity in shells of *O. lactea* and the lowest species diversity in shells of *T. pisana* (note that *C. aspersum* and *Iberellus* sp. were not analysed as there were no species with $n \geq 2$) (Table 3). The studied assemblages displayed significant differences in dominance, with the highest values found in shells of snail species that hosted species-poor assemblages, i.e., in *C. aspersum*, *Iberellus* sp. and *T. pisana* ($p < 0.05$, each, by bootstrapping; Table 3). The equitability and Fisher's alpha were the highest in *O. lactea* ($p < 0.05$ by bootstrapping; Table 3). The highest values of the Berger-Parker dominance index were in shells of snail species that hosted species-poor assemblages, i.e., in *C. aspersum*, *Iberellus* sp. and *T. pisana* ($p < 0.05$, each, by bootstrapping; Table 3). The Sørensen similarity indices indicated that there was low similarity among the assemblages that were associated with shells of individual shell species (Table 3).

*Cernuella* spp. (mostly *Cernuella virgata*) comprised about one half of the number of naturally present shells collected across sites (3,146; 50.1 %), followed with *Eobania vermiculata* (1,097; 17.5 %) and *Sphincterochila candidissima* (Draparnaud, 1801) (1,006; 16.0 %). *Iberellus* sp., *Monacha* spp. and *Pomatias elegans* were collected in less than 100 shells. *S. candidissima* hosted the highest number of nests (54 with 160 individuals; shell occupancy was 5.4 %); these nests were occupied by 14 species of bees and wasps. The same number of bee and wasp species was also hosted by shells of *E. vermiculata*. Nevertheless, the numbers of nests and individuals were lower in this shell type (42 nests with 71 individuals; shell occupancy 3.8 %). Nine species were recorded in shells of *Cernuella* spp. (27 nests with 35 individuals; shell occupancy 0.9 %) and in shells of *Otala lactea* (10 nests with 32 individuals; shell occupancy 3.5 %). Thus, the shells of *S. candidissima* were occupied by nesting species of bees and wasps preferentially compared to shells of other gastropod species. However, there were species-specific differences in shell preferences. Shells of *S. candidissima* were frequently occupied by *Osmia ferruginea*, *Hoplitis fertoni* (Pérez, 1891) and *Rhodanthidium sticticum*. Shells of *E. vermiculata* were frequently occupied by *Osmia andrenoides* Spinola, 1808, *O. aurulenta*, *O. ferruginea*, *Rhodanthidium septemdentatum* (Latreille, 1809) and *R. sticticum*. Shells of *Cernuella* spp. were frequently occupied by *O. ferruginea*. Two small species *Osmia versicolor* Latreille, 1811 and *Protosmia glutinosa* (Giraud, 1871) were recorded only in shells of *Cernuella* spp. but only in four and two nests respectively (Table 4).

**Table 3**
Species composition, species richness and diversity bees and wasps nesting in empty, naturally present snail shells. The selected species diversity indices are indicated.

| Snail species Index | *Cernuellavirgata* (Da Costa, 1778) | *Cornu aspersum* (O. F. Müller, 1774) | *Eobania vermiculata* (O. F. Müller, 1774) | *Iberellus* sp. | *Otala lactea*l (O. F. Müller, 1774) | *Sphincterophila candidissima* (Draparnaud, 1801) | *Theba pisana* (O. F. Müller, 1774) |
|---|---|---|---|---|---|---|---|
| Number of bee and wasp nests found | 28 | 1 | 43 | 2 | 11 | 57 | 5 |
| Number of bee and wasp species found | 10 | 1 | 15 | 2 | 9 | 15 | 2 |
| Chao-1 ± SD | 10.9 ± 2.8 | N/D | 27.7 ± 12.9 | N/D | 34.5 ± 19.3 | 27.7 ± 13.1 | 2.0 ± 0.5 |
| Dominance | 0.210 | N/D | 0.142 | N/D | 0.141 | 0.211 | 0.680 |
| Equitability | 0.840 | N/D | 0.846 | N/D | 0.955 | 0.755 | 0.722 |
| Fisher's alpha | 5.570 | N/D | 8.181 | N/D | 23.15 | 6.635 | 1.235 |
| Berger-Parker dominance index | 0.390 | N/D | 0.279 | N/D | 0.273 | 0.404 | 0.800 |
| Sørensen similarity index: | | | | | | | |
| - *Cornu aspersum* vs indicated cohort | 0.20 | | | | | | |
| - *Eobania vermiculata* vs indicated cohort | 0.44 | 0.13 | | | | | |
| - *Iberellus* sp. vs indicated cohort | 0.36 | 0.00 | 0.25 | | | | |
| - *Otala lactea* vs indicated cohort | 0.33 | 0.20 | 0.52 | 0.18 | | | |
| - *Sphincterophila candidissima* vs indicated cohort | 0.61 | 0.13 | 0.64 | 0.25 | 0.70 | | |
| - *Theba pisana* vs indicated cohort | 0.36 | 0.67 | 0.25 | 0.50 | 0.36 | 0.25 | |

### 3.5. Snail shell preferences by nesting species – experimentally deployed shells

In deployed shells, the most frequently used were the shells of *E. vermiculata*, in which we found thirteen individuals in seven nests of four species. Most of the nests were settled by *O. aurulenta*, which was also the only species nesting in shells of *C. aspersum* (10 individuals in four nests). Most species (six) settled the shells of a mid-sized snail species, *C. virgata*, however most were represented by only a single nest with one or several specimens (eight nests and 11 individuals). Importantly, there were no species recorded in shells of *Pomatias elegans*. When comparing the occupancy of naturally present and experimentally deployed shells, there were no differences in the occupancy of shells of *Cernuella* spp. ($\chi^2 = 1.724$, $d_f = 1$, $p > 0.05$), *C. aspersum* ($\chi^2 = 0.209$, $d_f = 1$, $p > 0.05$) and *P. elegans* (not tested; bees and wasps absent from both naturally present and experimentally deployed shells of this species). However, more nests of bees and wasps were present in naturally present shells of *E. vermiculata* when compared to the shells of the same species that were experimentally deployed ($\chi^2 = 8.032$, $d_f = 1$, $p = 0.005$).

Regarding the numbers of shells available for nesting at the locality, we found the highest numbers of species and nests at localities with a moderate number of empty shells (123 nests with 304 individuals of 21 species), followed by localities with low (17 nests with 28 individuals of 9 species) and high (11 nests of 11 individuals of 7 species) numbers of empty shells ($\chi^2$ test $p < 0.001$ for nests, individuals, and species, respectively, when compared to random distribution; see Fig. 2A for detrended correspondence analysis). The results of marked shells were affected by low numbers of nests in the deployed shells. There was a trend towards the higher occupancy rate of sites with low numbers of naturally present shells (10/17/6), which were followed by sites with moderate (5/6/3) and high (4/11/2) numbers of naturally present empty shells. However, the differences in occupancy rates were not significant (for detrended correspondence analysis, see Fig. 2B).

## 4. Discussion

The most surprising result of our survey is the very low number of bee and wasp nests that were recorded in deployed shells. Although we deployed 1,920 empty shells to the localities, only 17 of them (0.89 %) contained a bee or a wasp nest. This proportion was much lower than the number of deployed shells in similar study in central Europe where around 9% of the shells contained a hymenopteran nest (Heneberg et al., 2020). Only the shells collected randomly at the localities in central Europe contained a slightly lower proportion of nests, around 0.8 % (Bogusch et al., 2019) while in our study the number of settled, randomly collected shells was higher (150 nests in 6,276 shells; 2.39 %). The real difference is probably larger because the methodology used in the study of Heneberg et al. (2020) was less successful in finding shells, and many of the remaining shells could have been settled by species of bees, which hide the shells in the ground (i.e., *O. rufohirta* and *Osmia bicolor* (Schrank, 1781) in Central Europe), under stones and other materials present at the locality. In contrast, we found 2,174 of 2,720 total deployed shells (79.85 %), a much higher proportion, which means, that only every fifth shell remained uncollected at the locality or was destroyed when deployed at the locality. There was a slight difference among the retrieval of shells from different snail species, whereby the lowest rate concerned *Cernuella* spp. (504 found shells; 74.12 %), followed by 546 (80.29 %) of *P. elegans*, 551 (81.03 %) of *C. aspersum*, and 571 (83.97 %) of *E. vermiculata*. These results are very similar to those of Heneberg et al. (2020) who also found more shells of larger species. Shells of *Cernuella* spp. are often utilized by smaller species of *Osmia* and many of these species move and hide them (Müller et al., 2018). This fact can explain the lowest number of deployed shells of *Cernuella* spp. found at the localities. The proportions of shells among the localities did not differ very much while the lowest number was

**Table 4**
Species-specific associations of bees and wasps that were present in naturally present empty snail shells. The data are shown as the numbers of nests in shell of each snail species (the nest was counted once for each bee or wasp species recorded). The observed abundances were compared to expected random abundances by $\chi^2$ test, with the expected random data adjusted to the species-specific numbers of shells collected.

| Snail species Bee or wasp species | *Cernuella virgata* | *Cornu aspersum* | *Eobania vermiculata* | *Iberellus* sp. | *Otala lactea* | *Sphincterophila candidissima* | *Theba pisana* | Unknown | Total | $\chi^2$ test (vs. random) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Anthrax aethiops* (Fabricius, 1781) | 0 | 0 | 2 | 0 | 1 | 3 | 0 | 0 | 6 | 2E-219 |
| *Dioxys moesta* Costa, 1883 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| *Hoplitis fertoni* (Pérez, 1891) | 3 | 0 | 0 | 0 | 1 | 6 | 0 | 1 | 10 | 1.2E-127 |
| *Chrysis* sp. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Chrysura dichroa* (Dahlbom, 1854) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Chrysura hybrida* (Lepeletier, 1806) | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0 |
| *Chrysura rufiventris* (Dahlbom, 1854) | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 3 | 0 |
| *Leptochilus alpestris* (Saussure, 1856) | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 3 | 0 |
| *Osmia andrenoides* Spinola, 1808 | 1 | 1 | 3 | 0 | 1 | 1 | 1 | 0 | 8 | 3.1E-162 |
| *Osmia aurulenta* (Panzer, 1799) | 0 | 0 | 4 | 0 | 1 | 0 | 0 | 0 | 5 | 5.5E-265 |
| *Osmia fallax* Pérez, 1895 | 2 | 0 | 1 | 1 | 0 | 3 | 0 | 0 | 7 | 7.9E-187 |
| *Osmia ferruginea* Latreille, 1811 | 11 | 0 | 8 | 1 | 1 | 23 | 4 | 3 | 48 | 1.53E-12 |
| *Osmia rufohirta* (Latreille, 1811) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | |
| *Osmia versicolor* Latreille, 1811 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| *Priocnemis propinqua* (Lepeletier, 1847) | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0 |
| *Protosmia glutinosa* (Giraud, 1871) | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 4 | 0 |
| *Rhodanthidium infuscatum* (Erichson, 1835) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| *Rhodanthidium septemdentatum* (Latreille, 1809) | 1 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 5 | 5.7E-265 |
| *Rhodanthidium sticticum* (Fabricius, 1878) | 0 | 0 | 12 | 0 | 3 | 9 | 0 | 4 | 24 | 2.17E-44 |
| *Sapyga quinquepunctata* (Fabricius, 1781) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Stenodynerus* sp. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Trichodes apiarius* (Linnaeus, 1758) | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 0 |
| *Trachusa pubescens* (Morawitz, 1873) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| N/D | 2 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 8 | 3.2E-162 |

found in clayish embankment (locality I1) (44.38 %) where the clay sediment probably covered the shells during the season.

In the collected shells, there were big differences in the occupancy among the localities while the highest numbers of occupied shells were in the localities in the dry area: D1 with 177 individuals of 11 species in 61 nests (11.23 % of shells occupied), followed by D4 (62 individuals of 10 species in 24 nests; 4.26 % shells occupied). This may correspond with the fact that all neighbouring habitats of orchards in dry regions were of near-natural character with a number of native flowering plants. Only the locality W1 in a wet region hosted quite good proportion of nests, 30 individuals of nine species in 19 nests (2.94 %). In several localities, we recorded a lower number of nests but the total number of shells collected was significantly lower due to very small number of shells at the locality: D3 with 17 individuals of five species in seven nests (occupancy 9.33 %) and D2 with 31 individuals of seven species in 14 nests (occupancy 4.68 %). Shells in all other localities hosted only several nests with occupancy lower than 1%. Moreno-Rueda et al. (2008) recorded 122 bee individuals in 3,600 shells of *S. candidissima*, which means 3.34 % of shells occupied. The occupancy of shells of this species is lower than in our study (5.37 %) but generally agrees with our results.

We recorded species commonly known to nest in empty gastropod shells, including the most common shell-nesters known from this region, led by *O. ferruginea*, *R. sticticum*, *O. aurulenta*, *O. andrenoides*, *H. fertoni* and *Osmia fallax* Pérez, 1895 (Müller, 1994; Le Goff, 2003; Moreno-Rueda et al., 2008; Müller et al., 2018). The spider wasp *Priocnemis propinqua* Lepeletier, 1847 was not known to place its nests in empty gastropod shells and we recorded two nests at two localities. This species hunts for terrestrial spiders, especially of the family Gnaphosidae (Wolf, 1971), and puts the paralyzed spiders into various cavities. Snail shells can be sparsely used too. Bogusch et al. (2019) recorded for the first time two species of spider wasps in empty shells in central Europe - both generalists (*Anoplius nigerrimus* (Scopoli, 1763) and *Auplopus carbonarius* (Scopoli, 1763)). One individual of *Trachusa pubescens* (Morawitz, 1873) was recorded in a shell. This species places its nest underground, similar to other species of this genus (Kasparek, 2017) so the specimen probably only used the shell in order to avoid bad weather. A new host record is the cuckoo bee *Dioxys moesta* Costa, 1883, which was formerly known to invade nests of several species of *Osmia* and *Hoplitis* (Warncke, 1977). Our specimens were reared from nests of *H. fertoni* and this bee is newly confirmed host of *D. moesta*.

The naturally occurring snail shells density at the localities was quite high, often reaching around 40–80 shells per square meter. Generally the slopes with ruderal vegetation hosted very rich populations of medium-sized (*Cernuella* or *Theba*) or large (*Eobania* and *Otala*) snail species while the near-natural habitats in dry regions contained much lower number of shells and bigger snail species (*Sphincterochila*, *Eobania* and *Otala*) were most numerous. This fact can be connected with the explanations of why the deployed shells were settled only by a small number of nests: the bees had many available shells at the locality and thus did not need to settle the shells, which were experimentally deployed. Moreover, ruderal sites are much poorer in flowering plant species and thus they host less species of bees than near-natural sites. Several of the bees nesting in empty gastropod shells have very specific nesting biologies, settling only shells in rock or wall cavities or placed under stones. This nesting biology is typical for the genus *Rhodanthidium* (L. Hlaváčková & P. Bogusch, unpublished data) and our results correspond with their nesting biology very well. Both *R. septemdentatum* and *R. sticticum* are numerous in the studied region (Torné-Noguera et al., 2014) and *R. sticticum* was frequently recorded in collected shells. However, we did not record these species nesting in experimentally deployed shells although (L. Hlaváčková & P. Bogusch, unpublished data) recorded plenty of nests of both species of this genus under stones and in rock and wall cavities.

Finally, we confirmed that high proportion of gastropod shells at the localities is essential for many bees, which depend on the habitat for nesting and food, both as adults and larvae (Westrich, 2018; Westerfelt et al., 2018). Near-natural habitats provide abundant shells for nesting bees, with a plentiful amount of pollen and nectar available both from the surrounding native plants and the blossoms in the nearby orchards. Thus, these near-natural sites, as well as small patches with ruderal vegetation, are very important for shell-nesting bees, which can benefit from this situation with nesting and food resources in a small area. Although the landscape in Catalonia is less influenced by human activities than the landscape in central Europe, the near-natural sites around fruit orchards may serve as biodiversity hotspots for bees, similar to small field wetlands in central Europe (Heneberg et al., 2018). Further surveys will help to confirm the importance of these habitats in the South-European landscape. In addition, studies on bees directly pollinating almond and other trees in orchards will demonstrate whether the bees nesting in empty shells represent a large abundance of the native pollinators.

## 5. Conclusions

Small to medium-sized near-natural habitat patches neighbouring almond orchards are very important habitats in Southern European landscapes for both bees and wasps. We confirmed that these habitats host strong populations of bees nesting in empty shells of terrestrial gastropods, these gastropod species being very numerous in these habitats, especially in dry regions. Similar microhabitats of ruderal character are inhabited by less abundant and less species-rich assemblages of these bees. Fruit trees are dependent on insect pollination and it is essential that consideration is given to native pollinators, not simply the commercial honeybees and bumblebees that are kept in hives near orchards. Thus, the presence of microhabitats of natural character near the orchards is very important both for the crops and the sustainability of the habitats.

Bees and wasps nesting in empty gastropod shells usually prefer the larger and medium-sized species (*S. candidissima*, *E. vermiculata* or *Cernuella* spp.), which are numerous at the localities. There are big differences in the preferences of shells among the bee and wasp species, and also in the nesting habits. These differences can help the species inhabit their biotopes - more species can find a habitat at quite small but heterogeneous sites because of a greater species richness of shell being present. The use of experimentally deployed shells showed that the bees and wasps found a portion of shells suitable to nest in the localities and could therefore form strong populations. Neighbouring almond trees may provide additional sources of nectar and pollen.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2020.106949.

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V rámci našich výzkumů na přírodě blízkých lokalitách u mandloňových sadů nedaleko města Lleida ve Španělsku jsme získali větší množství hnízd dvou druhů samotářských včel – *Hoplitis fertoni* a *Osmia ferruginea*. Podrobnosti o hnízdění těchto dvou druhů doposud nebyly příliš prozkoumány.

Převážná většina ulit s hnízdy byla sesbírána na lokalitách v suché kopcovité oblasti, pouze několik ulit druhu *O. ferruginea* bylo v oblasti říční nivy. Ulity s hnízdy obou druhů byly nalezeny na povrchu země a nebyly ničím zakryté. Nejvíce hnízd bylo v ulitách druhu *Sphincterochila candidissima*.

Popsali jsme hnízdní strukturu těchto dvou druhů. Oba druhy staví v hnízdě větší množství plodových buněk, toto množství je ale variabilní a odvíjí se od velikosti dané ulity. *H. fertoni* využívá ke stavbě zátky i přepážek jíl a stejným materiálem vykládá také stěny hnízdních komůrek. Za zátkou je někdy vystavěna prázdná komůrka, jindy ovšem chybí. Některá hnízda mohou mít prázdnou komůrku uvnitř hnízda mezi plodovými buňkami. Komůrky jsou většinou umístěny lineárně za sebou, u některých hnízd však bylo v prostoru u ústí ulity nalezeno několik komůrek vystavěných vedle sebe. Oproti tomu *O. ferruginea* využívá ke stavbě hnízda rozžvýkaný rostlinný materiál, a to jak na zátku, tak i na přepážky. Stěny plodových komůrek tímto materiálem nevykládá, ani jím nepotírá povrch ulity. Za zátkou bývá prázdná komůrka a plodové komůrky jsou umístěny lineárně.

Některé ulity byly parazitované, a to převážně druhy, které doposud nebyly u těchto včel zaznamenány. Nejčastějším parazitem u *H. fertoni* byla zlatěnka *Chrysura hybrida*, mimo ni jsme v hnízdech nalezli také kodulky *Stenomutilla collaris* a *S. hotentotta,* kukaččí včelu *Dioxys moesta* a dlouhososku *Anthrax aethiops*. Hlavním parazitem u druhu *O. ferruginea* byla drvenka *Sapyga quinquepunctata*, dále byla nalezena i dlouhososka *A. aethiops*.

Na základě pylových analýz jsme popsali pylovou specializaci obou druhů samotářských včel. *H. fertoni* je druh oligolektický na Boraginaceae, *O. ferruginea* polylektický sbírající pyl ponejvíce na rostlinách čeledí Cistaceae, Fabaceae a Lamiaceae.

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# Nest structure, pollen utilization and parasites associated with two west-Mediterranean bees (Hymenoptera, Apiformes, Megachilidae) nesting in empty snail shells

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## Abstract

Around thirty species of European solitary bee species in the family Megachilidae nest in empty gastropod shells. We surveyed this group of bees in semi-natural sites adjacent to almond orchards near Lleida (north-eastern Spain) and collected 35 *Hoplitis fertoni* and 58 *Osmia ferruginea* nests in shells of six snail species. We describe the nest structure and report the identity of pollens collected by the two bee species. Both species adjust the number of brood cells to the size of the shell and occasionally build intercalary (empty) cells. *H. fertoni* uses clay and *O. ferruginea* chewed plant leaves for building cell partitions and nest plugs. Most nests of both species were built in *Sphincterochila candidissima* shells. Analysis of the pollen of selected nests confirmed that *H. fertoni* is oligolectic on Boraginaceae (in our study all pollen was from *Lithodora fruticosa*) and *O. ferruginea* is a polylectic species (collecting mostly pollen from Cistaceae, Fabaceae, and Lamiaceae in our study area). Nests of *H. fertoni* were parasitized by five species, the golden wasp *Chrysura hybrida*, the cuckoo bee *Dioxys moesta*, the velvet ants *Stenomutilla collaris* and *Stenomutilla hotentotta*, and the bee-fly *Anthrax aethiops*; nests of *O. ferruginea* were parasitized by the sapygid wasp *Sapyga quinquepunctata* and *A. aethiops*. Except for *C. hybrida* these are newly recorded host-parasite associations. Our results confirm previous information and bring new findings on the ecology of both species.

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**Keywords**
Spain, Lleida, *Hoplitis fertoni*, *Osmia ferruginea*, parasitoid, cleptoparasite, pollen specialization

## Introduction

Bees (Anthophila/Apiformes) are a very speciose clade of Hymenoptera, with more than 20,000 species worldwide (Michener 2007). Most of these species are solitary and build their nests underground (Michener 2007; Danforth et al. 2019). However, about a third of the solitary bee species nest above-ground, mostly in pre-established cavities (Bogusch and Horák 2018). Among these, a small group of species have specialized in nesting in empty gastropod shells. This behaviour is widespread in the Old World (Müller et al. 2018), including the Palaearctic and Southern Africa (Gess and Gess 1999, 2008), but much rarer in the New World (Michener, 2007). In Europe about 30 species in the genera *Osmia*, *Hoplitis*, *Protosmia* and *Rhodantidium* are known to construct nest in gastropod shells. The majority of these species have distributions restricted to the southernmost part of the continent (Müller 1994; Müller et al. 2018). Most of these species are very specialized in their nesting substrate choice and rarely use other cavity types (but see Bosch et al. 1993). The nesting biology of species occurring in central Europe is fairly well-known. Several species display a series of behaviours related to the manipulation, translocation, and camouflaging of the nest shells (Bellmann 1981; Müller et al. 2018). Various species of *Chrysura* golden wasps have been reported as parasitoids of these species (Müller 1994; Westrich 2018; Bogusch et al. 2019). However, the nesting biologies of species occurring only in southern Europe remain poorly known (Müller et al. 2018). During our studies on shell-nesting bees near Lleida (Catalonia) in northeast Spain we collected a good number of nests of *Hoplitis fertoni* and *Osmia ferruginea*, two species with a Mediterranean distribution.

*Hoplitis fertoni* occurs in North Africa, Spain, Portugal, and Sicily, and may be locally abundant. It builds its nests in shells of large snail species (such as *Eobania*, *Otala* and *Theba*), and uses mud to build brood cell partitions and to close the nest (Ferton 1908; Le Goff 2003; Müller et al. 2018). *H. fertoni* nests in spring and does not move or cover its nesting shells (Le Goff 2003). Cuckoo wasps *Chrysura hybrida*, *Chrysura cuprea* and *Chrysura trimaculata* are parasitoids of *Hoplitis fertoni* and several other bees nesting in empty shells (Berland and Bernard 1938; Petit 1969, 1980; Le Goff 2003; Wiśniowski 2014).

*Osmia ferruginea* occurs in all countries around the Mediterranean and may be locally common in the West-Mediterranean region (Müller 2019). It flies in the spring and utilizes shells of a high number of snail species of various sizes, where it builds 1–10 brood cells separated by partitions of masticated plant matter (Ferton 1905; Saunders 1908; Alfken 1914; Benoist 1931; Mavromoustakis 1952; Grandi 1961; Haesseler 1997; Moreno-Rueda et al. 2008). Although this species is widespread in South Europe, no associated parasitoid species are known (Müller 2019).

Here, we describe the nest structure of *Hoplitis fertoni* and *Osmia ferruginea*, and report on the snail shells used and the pollens collected by these two species. We also report on several parasitoids and nest cleptoparasites reared from the collected nests. We discuss our results in relation to previous information available for these two species (Ferton 1905, 1908; Benoist 1931; Grandi 1961; Haesseler 1997; Le Goff 2003; Moreno-Rueda et al. 2008; Müller et al. 2018; Müller 2019).

## Methods

In March 2019 we collected more than 500 large gastropod shells (the size of semiadult *Cernuella virgata*, 8 mm, or bigger) in 10 semi-natural sites with high shell availability around almond orchards near Lleida. We also collected 434 shells in three additional localities in which shell availability was lower. Some of the shells contained *Hoplitis fertoni* nests built in the previous year (with cocoons). Other shells contained fresh *Osmia ferruginea* nests (with provisions and eggs/larvae).

*Hoplitis fertoni* nests were dissected 0–8 days after collection. The number of brood cells, their shape and positions within the shell and the number of larvae, pupae and adults were recorded. Some remnants of pollen provisions were collected and placed inside plastic micro-tubes for later identification. Some *O. ferruginea* nests were also dissected 0–8 days after collection. The rest were dissected at biweekly intervals until May 11$^{th}$, when all nests contained mature larvae in cocoons (prepupae). All brood was kept under laboratory conditions until adult eclosion which occurred by September the same year. The cocoons were opened in September 2019, when all bees and their parasites developed into adults and were alive inside the cocoons, two specimens of the brood parasite *Sapyga quinquepunctata* spontaneously hatched and left the cocoons a few days before we opened them.

Pollen samples were prepared using a standard acetolysis method (Moore et al. 1991). Pollen were boiled for 5 min. in an acetolysis mixture of sulphuric acid ($H_2SO_4$) and acetic anhydride $(CH_3CO)_2O$ (1 : 9 ratio). Samples were then transferred to a mixture of water and glycerol. Slides were observed at 400× magnification. Pollen grains were determined using pollen identification keys (Punt and Clarke 1984; Moore et al.1991; Reille 1992; Beug 2004) and the reference collection of the Institute of Botany of the Czech Academy of Sciences.

Photos of shells containing nests, closing plugs, and dissected nests were taken with a digital camera Nikon Coolpix B500. Photos of larvae, brood cells, and nest details were taken with a digital camera Canon EOS 550 and a macro-objective equipped with LED goose-neck light. Final figures were created from multiple level-photos stacked by Zerene Stacker software. We drew figures of nest structure using pen-drawing and colouring in Adobe Photoshop. Photos of pollen grains were taken under a light microscope Delphi X-Observer DX 2153-PLi with a camera Moticam 5+ and software for photo analysis.

## Results

### *Hoplitis fertoni*

**Nest structure.** We collected 35 gastropod shells with nests of *H. fertoni* in three of the 13 localities surveyed. All of the localities were situated in dry hilly region. All shells were found on the ground surface and were not hidden. The closing plug was made of soil of light-brownish or greyish colour (Fig. 1A). In most nests (32) the closing plug was placed at the shell aperture. In the remaining three shells it was placed a few mm inside the shell. Several nests had a vestibular (empty) cell below the plug. The rest of the shell was filled with brood cells. Some nests had one or more empty intercalary cells. The brood cell walls were fully lined with soil and inter-cell partitions were double (Fig. 1D). The brood cells were arranged longitudinally along the spire of the shell cavity but some nests had two or more cells arranged transversally close to the nest aperture. Brood cell partitions were 2–5 mm thick (mean 2.6 mm) and the closing plug 3,5–8 mm thick (mean 4.9 mm) (Fig. 1D).

**Shell choice.** The majority (26, 74.3%) of the nests were built in shells of *Sphincterochila candidissima*. Other snail species used were *Eobania vermiculata* (4, 11.4%), *Cernuella* sp. (3, 8.6%) and *Otala lactea* (1, 2.9%). The 35 nests collected contained 217 brood cells (mean ± SD: 6.2 ± 2.24; range: 2–10 brood cells per nest). The nests in *S. candidissima* shells contained 4–9 brood cells (mean 6.4, median 7), and those in *E. vermiculata* shells 5–10 brood cells (mean 5.8, median 6). Nests in the smaller *Cernuella* sp. shells contained fewer cells (range 2–3, mean 2.3, median 2).

**Nest associates.** Altogether 58 (26.7%) brood cells contained dead, dry or mouldy contents. Of the remaining brood cells, 126 contained pupae or adults of *H. fertoni*, and 33 were parasitized (25.8% of brood cells containing live insects). The golden wasp *Chrysura hybrida* (Chrysididae) was the most common parasitoid (21 cells in 14 nests). Cells parasitized by *C. hybrida* were recognizable by the presence of a semi-transparent brownish cocoon with a whitish spot within the thicker brownish cocoon of *H. fertoni*. We also found five nests parasitized by the velvet ant *Stenomutilla collaris* (seven cells) and one nest by *Stenomutilla hotentotta* (one cell) (Mutillidae). Velvet ants pupated and became adults by late spring (late May – June). *Stenomutilla* cocoons were very similar to those of *C. hybrida* but harder and darker and did not have whitish marks. We also found three nests parasitized by the cuckoo bee *Dioxys moesta* (Megachilidae) (one cell per nest). The cocoons of this species were composed of a single whitish layer sparsely covered with dark brownish faecal particles. Pupation and adult eclosion occurred more or less at the same time as in *H. fertoni*. Finally, we found one nest with one cell parasitized by the bee-fly *Anthrax aethiops* (Bombyliidae). The structure of all nests is illustrated in Fig. 2.

**Pollen contents.** We analysed pollen samples (remnants of unconsumed provisions) from six nests from two localities (S35 and S37). All pollen grains identified were *Lithodora fruticosa* (Boraginaceae) (Fig. 3A). During of March 2019 we repeatedly observed *Hoplitis fertoni* females collecting pollen only on flowers of this species in various localities.

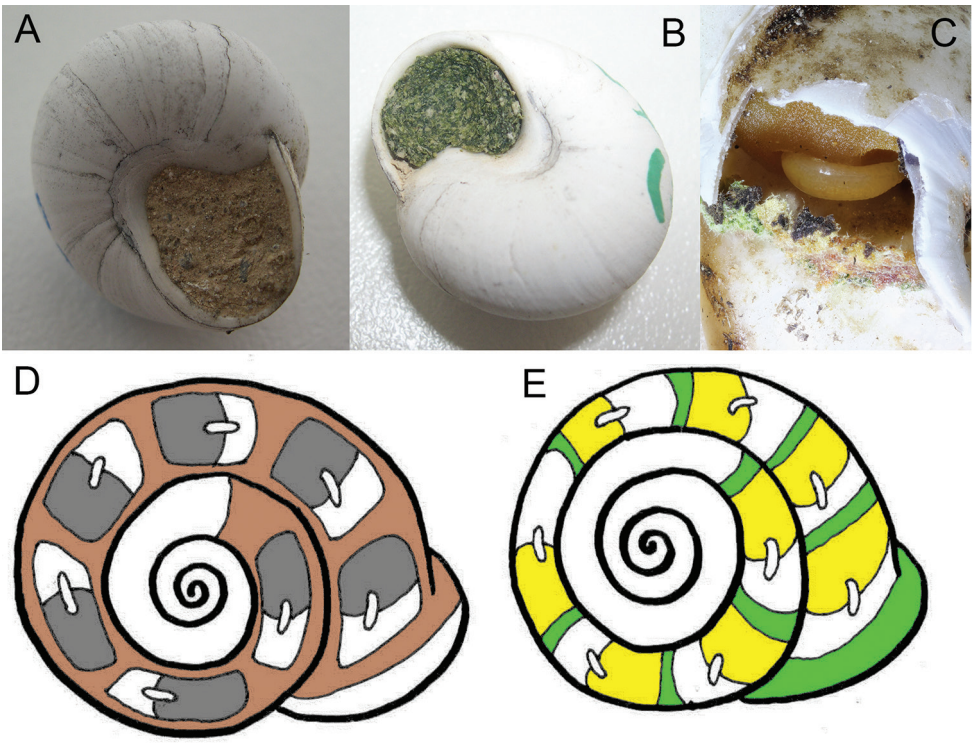


**Figure 1.** Structure of *Hoplitis fertoni* (**A, D**) and *Osmia ferruginea* (**B, C, E**) nests **A** shell of *Sphinctero-chila candidissima* with nest of *H. fertoni* **B** shell of *S. candidissima* with nest of *O. ferruginea* **C** larva of *O. ferruginea* on pollen-nectar provision **D** nest structure of *H. fertoni* **E** nest structure of *O. ferruginea*. Photos and drawings by P. Bogusch.

### *Osmia ferruginea*

**Nest structure.** We collected 58 shells with nests of *O. ferruginea* in nine of the 13 localities surveyed. Most nests (48) were collected in the localities of a dry hilly area. The remaining 10 nests were collected in the river floodplains. All nests were found exposed (not hidden) at ground level. The surface of the shells had no traces of masticated leaf matter. The closing plug was made of green masticated leaf matter (Fig. 1B). In most nests (38) it was placed at the shell aperture, but in some it was placed inside the shell. All nests had a vestibular (empty) cell below the plug. Brood cells were separated by narrow single partitions of masticated plant matter and the side walls of the brood cells were not lined (Fig. 1C). At the time nests were dissected (March 2019), each cell contained a yellow to light yellow spherical pollen provision with an egg or a young larva (Fig. 1C). The brood cells were always placed longitudinally along the spire of the shell. Brood cell partitions were around 1 mm thick and the closing plug 1.5–3 mm thick (mean 2.1 mm) (Fig. 1E).

**Shell choice.** Most nests (28, 48.3%) were built in *Sphincterochila candidissima* shells. The remaining nests were placed in shells of *Eobania vermiculata* (13, 22.4%),

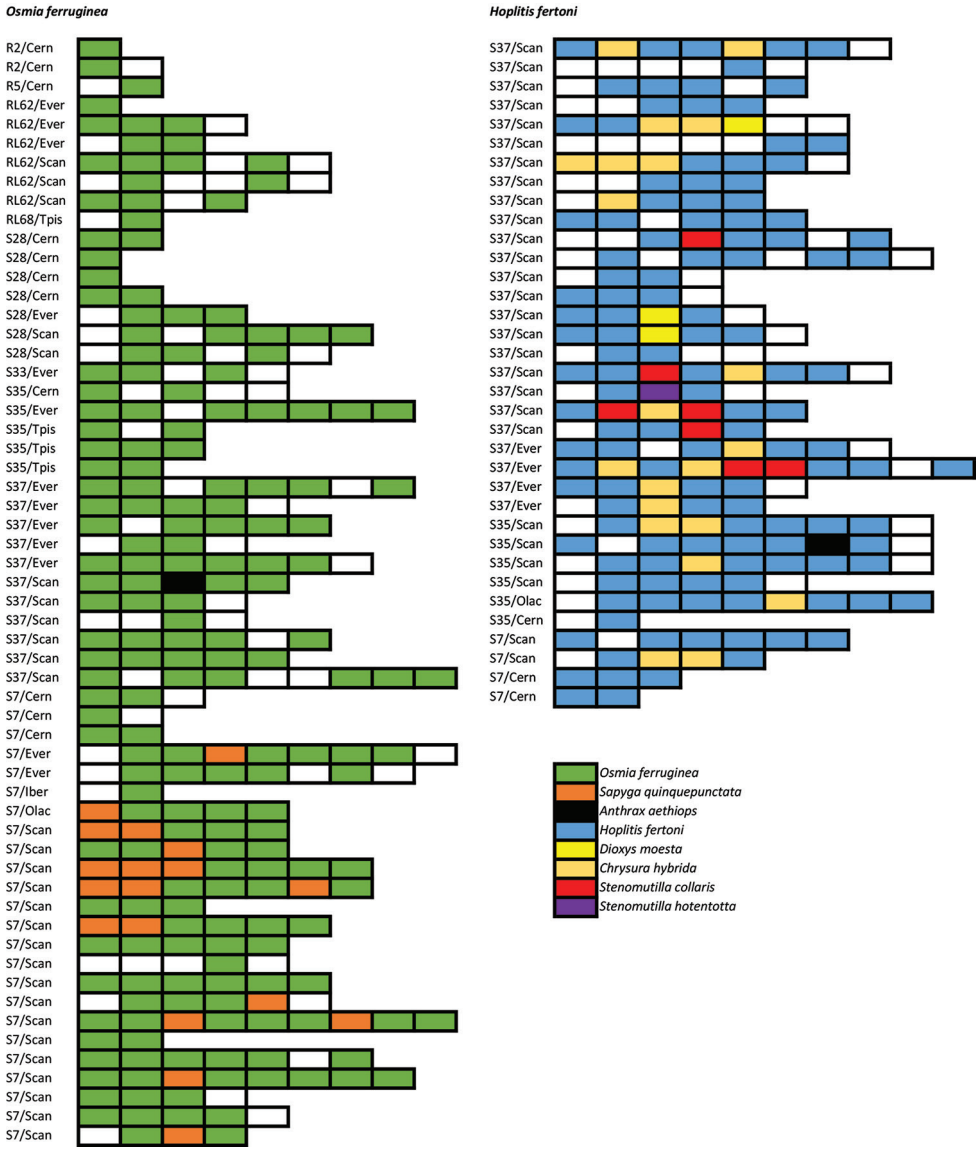


**Figure 2.** Diagrams of nest structures of nests of *Hoplitis fertoni* (right) and *Osmia ferruginea* (left). The nests are identified by locality snail codes (Cern – *Cernuella* sp., Ever – *Eobania vermiculata*, Iber – *Iberellus* sp., Olac – *Otala lactea*, Scan – *Sphincterochila candidissima*, Tpis – *Theba pisana*. Each box represents one brood cell, starting with the innermost cell on the left. Colours represent the various species recorded. White boxes represent intercalary cells.

*Cernuella* sp. (11, 18.9%), *Theba pisana* (4, 6.9%), *Iberellus* sp. (1, 1.7%) and *Otala lactea* (1, 1.7%). The 58 nests collected contained 268 brood cells (mean ± SD: 4.6 ± 2.09; range: 1–9 brood cells per nest). The nests in *S. candidissima* shells contained 2–9 brood cells (mean 5.6, median 5), and those in *E. vermiculata* shells were similar 1–9 brood cells (mean 5.5, median 6). Nests in the smaller shells of *Cernuella* sp. and

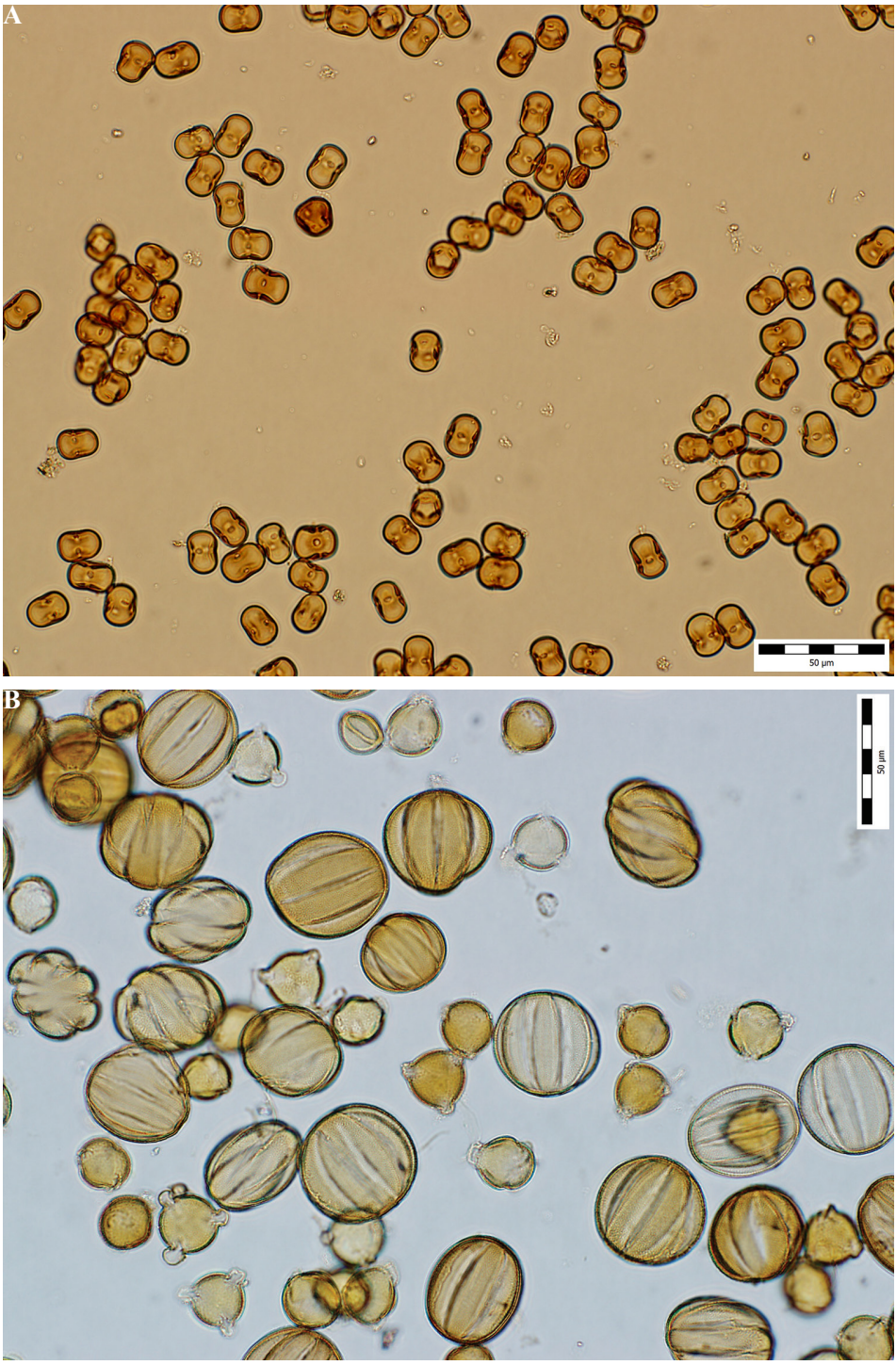


**Figure 3. A** Macrophotography of pollen of *Lithodora fruticosa* from a *Hoplitis fertoni* nest (locality S35). Photo by L. Petr. **B** macrophotography of pollen from an *Osmia ferruginea* nest (locality S7). Larger pollen grains are *Thymus vulgaris*; smaller grains are *Cistus albidus*. Photo by L. Petr.

**Table 1.** Pollen composition of six provision samples from *Osmia ferruginea* nests.

| Site | Pollen contents |
|---|---|
| S35 | *Cistus albidus* (Cistaceae) ca. 100%, Asteraceae and *Thymus vulgaris* (Lamiaceae) |
| S37 | *Cistus albidus* (Cistaceae) 37 %, *Cytisus scoparius* (Fabaceae) 37 %, *Olea europaea* (Oleaceae) 26 % |
| S37 | *Cistus albidus* (Cistaceae) 50%, *Cytisus scoparius* (Fabaceae) 40%, *Olea europaea* (Oleaceae) 10% |
| S7 | *Cistus albidus* (Cistaceae) 48 %, *Cytisus scoparius* (Fabaceae) 27 %, *Olea europaea* (Oleaceae) 22 %, *Thymus vulgaris* (Lamiaceae)12 %, contamination of three pollen types of Asteraceae |
| S7 | *Thymus vulgaris* (Lamiaceae) 51 %, *Cytisus scoparius* (Fabaceae) 49 % |
| S7 | *Cytisus scoparius* (Fabaceae) 77 %, *Thymus vulgaris* (Lamiaceae) 23 % |

*T. pisana* contained fewer cells (range 1–5, mean 2.1, median 2 and range 2–3, mean 2.5, median 2–3, respectively).

**Nest associates.** Altogether 56 (20.9%) of the brood cells contained dead, dry or mouldy contents. Most of the remaining brood cells (203) contained pupae or adults of *O. ferruginea*, while 19 contained parasitoids (9% of brood cells containing alive insects). The main parasitoid species was *Sapyga quinquepunctata* (Sapygidae; 18 cells from 11 nests, all from locality S7; 19% parasitism). All the individuals of this cleptoparasitic species reached adulthood by late summer or beginning of autumn (September). Brood cells parasitized by *S. quinquepunctata* were recognizable by the dark brown oval-shaped cocoon, distinct from the cubic cocoons of *O. ferruginea*. The bombyliid *Anthrax aethiops* was recorded in a single cell of one nest. The structure of all nests is illustrated on Fig. 2.

**Pollen contents.** We analysed six pollen samples from nests collected at three different localities. Most pollen grains were of Cistaceae, Fabaceae (*Cytisus* type), and Lamiaceae (Table 1; Fig. 3B).

## Discussion

Nest structures of both species correspond to the nest descriptions published by previous authors (Ferton 1905, 1908; Benoist 1931; Grandi 1961; Haesseler 1997; Le Goff 2003; Moreno-Rueda et al. 2008; Müller et al. 2018; Müller 2019). Both *Hoplitis fertoni* and *Osmia ferruginea* belong to the group of species building high numbers of brood cells per nest. Most bee species nesting in empty gastropod shells build low numbers of brood cells per shell (usually only one or two) (see Müller et al. 2018). The number of brood cells per nest in both *Hoplitis fertoni* and *Osmia ferruginea* is highly variable depending on the size of the shell utilized. Compared to other middle-sized bee species such as *Osmia rufohirta*, which usually nests in shells of *Xerolenta obvia* and builds one brood cell per nest (Bellmann 1981; Müller et al. 2018; Heneberg et al. 2020), *Hoplitis fertoni* and *Osmia ferruginea* seem to prefer bigger shells (specially *Sphincterochila candidissima*) and build a higher number of cells per nest. *S. candidissima* has also been reported as the most commonly used shell in other studies on shell-nesting bees (Moreno-Rueda et al. (2008), Bogusch et al. (in press).

The number of parasitic species associated with *H. fertoni* (5) in our study is remarkable. The most common parasitoid, *Chrysura hybrida* was already recorded on *H. fertoni* by Le Goff (2003). This species has been associated to several other Osmiini, nesting either in snail shells (*Osmia versicolor*, *Osmia viridana*) or in other types of cavities (*Hoplitis anthocopoides*, *Hoplitis benoisti*, *Hoplitis ravouxi*, *Osmia caerulescens*, and *Anthocopa villosa*) (Berland and Bernard 1938; Grandi 1961; Petit 1969, 1980). Most of these records are based on observations of the bee and the wasp co-occurring at a given locality but, together with our findings, they suggest that *C. hybrida* specializes on smaller Osmiini, irrespective of the nesting substrate. Other species of *Chrysura* also parasitize bees of the family Megachilidae, and many of them appear to show a strong preference for bees nesting in snail shells (see Müller et al. 2018).

We also reared two species of velvet ants from the nests of *H. fertoni*. Velvet ants are ectoparasitoids and usually have a broad host spectrum. Some species preferentially parasitize either bees or wasps, but others have been recorded on both guilds of hymenopterans (Lelej 1985; Brothers 1989; O'Neill 2001). The biology of *Stenomutilla* is not well-known, but several authors have reported associations with solitary wasps, megachilid bees and chrysomelid beetles of the subfamily Clytrinae (Giner Marí 1944; Brothers 1989). Thus, it is likely that both species of *Stenomutilla* recorded in our study parasitize a wide range of bees and possibly wasps. However, in a broader study in the same geographical area we have examined hundreds of nests of more than ten snail-nesting bees and we found *Stenomutilla* only in nests of *H. fertoni* (Bogusch et al., in press).

The bee-fly *Anthrax aethiops*, has been recorded as a parasitoid in nests of more than ten bee species, some of them nesting in gastropod shells (Austen 1937; Du Merle 1972; Peeters et al. 2012; Müller et al. 2018). Heneberg et al. (2020) found this species to be a frequent parasitoid of *Osmia spinulosa* and two other species nesting in gastropod shells. This parasitoid has probably a broad host spectrum mostly including megachilid bees but also other bees. Although this species has only been reported as a parasitoid of bees, many other representatives of this family are beside bees parasitoids of crabronid wasps (Yeates and Greathead 1997; Bogusch et al. 2015).

*Dioxys moesta* is a cuckoo bee occurring in south Europe and North Africa. Its host spectrum is unknown. Other *Dioxys* species in Europe and neighbouring regions, are cleptoparasitic on bees of the family Megachilidae, mostly *Hoplitis* and *Osmia* (Westrich 2018), but up to now they were not known to parasitize species nesting in shells. The question whether *D. moesta* specializes on shell-nesting species remains open.

*Sapyga quinquepunctata* is an unspecialized cleptoparasite in nests of several Megachilidae (Stöckhert 1933; Vicens et al. 1993; Vicens et al. 1994; Gusenleitner and Gusenleitner 1994; Osorio et al. 2018; Müller et al. 2018; Torné-Noguera et al. 2020). Although it has been reared from nests of *Osmia bicolor* (Westrich 2018; Heneberg et al. 2020), it does not appear to be common in nests of shell-nesting species (Bogusch et al. 2019; Heneberg et al. 2020). Our findings of *S. quinquepunctata* and *Anthrax aethiops* in nests of *O. ferruginea* represent the first records of nest parasitism in this species.

Analysis of the pollen provisions yielded contrasting results for the two species studied. Previous studies have reported *Echium* (Boraginaceae) as the only pollen

source of *H. fertoni* (Le Goff 2003, Sedivy et al. 2013; Müller 2019). Both pollen analysis and field observations indicate that in the study area this species visits another Boraginaceae, *Lithodora fruticosa*. Thus, with the information currently available, *H. fertoni* should be considered oligolectic on Boraginaceae, just like most species of the *Hoplitis adunca* species-group (Müller 2019). We have also observed flowering *L. fruticosa* at five localities studied, and three females of *H. fertoni* in three localities (one in each) were observed on flowers of this plant. No species of *Echium* was in flower during our studies (middle March 2019) so *L. fruticosa* is probably the only useful source of pollen for first females of *H. fertoni* provisioning their nests. In other regions, the situation can be different and this species can specialize on other pollen sources, especially of genus *Echium* (as was published by Le Goff 2003 and Müller et al. 2018). *H. fertoni* lacks any of the morphological adaptations usually present in bees that harvest pollen from flowers with included anthers, so it is a question how it is able to efficiently exploit these flowers. Perhaps some behavioral trick that might be revealed by detailed field observation. On the other hand, our results confirm that *O. ferruginea* is a polylectic species with preference for the Fabaceae (Müller 2019). In our nests, all provisions analyzed contained pollen from several plant families. Other species in the subgenus *Pyrosmia* are usually polylectic, but several are oligolectic on Fabaceae (Müller 2019).

The biology of shell-nesting bees from south Europe is poorly known. Our study contributes to filling this gap by providing new records of parasites and pollen use, as well life history and nesting behaviour traits. Interestingly, some of these traits are shared with bees of the same taxa nesting in other types of cavities. For example, *Hoplitis adunca* also lines cell walls, builds double inter-cell partitions and is oligolectic on Boraginaceae (Bosch et al. 2001). For the most part, parasites recorded in this study also seem to be shared by species nesting in other substrates. Nesting behaviour, pollen preferences and host-parasite associations are important sources of information for the reconstruction of phylogenies and for tracking the evolution of behavioural traits in Megachilid bees (Müller 1996; Bosch et al. 2001; Sédivy et al. 2008, Litman et al. 2011; González et al. 2019).

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**5.6 Hostinská L., Kuneš P., Hadrava J., Bosch J., Scaramozzino P., Bogusch P. 2021: Comparative biology of four *Rhodanthidium* species (Hymenoptera, Megachilidae) that nest in snail shells. *Journal of Hymenoptera Research* 85: 11–28. https://doi.org/10.3897/jhr.85.66544.**

Výzkumy evropských druhů rodu *Rhodanthidium,* které hnízdí v prázdných ulitách, probíhaly ve střední Evropě a ve Španělsku. Konkrétně se jedná o druhy *R. septemdentatum, R. sticticum, R. siculum* a *R. infuscatum*. Jejich hnízdní biologie doposud nebyla příliš známá, naše výzkumy tak přinesly nové informace o struktuře jejich hnízd, preferovaných druzích ulit, parazitech a pylové specializaci.

Výzkum probíhal dvěma metodami – vyhledáváním a sběrem zahnízděných ulit a v případě druhů *R. septemdentatum* a *R. sticticum* také přímým pozorováním hnízdících samic.

Všechny čtyři druhy si obvykle vybírají ulity větších velikostí (ve Španělsku to byl nejčastěji druh *Eobania vermiculata*, ve střední Evropě *Caucasotachea vindobonensis*) a ke stavbě hnízda využívají obdobný materiál (především písek, malé kamínky a fragmenty ulit spojené pryskyřicí). Struktura hnízda i počet hnízdních komůrek se však u jednotlivých druhů liší. Větší druhy, *R. septemdentatum, R. sticticum* a *R. siculum,* obvykle staví pouze jednu komůrku, někdy dvě. Oproti tomu menší *R. infuscatum* staví dvě až čtyři komůrky na hnízdo.

Všechna studovaná hnízda měla mezi zátkou a posledně vystavěnou plodovou buňkou dlouhou (vestibulární) komůrku. Hnízda jednotlivých druhů se pak lišila v přítomnosti či nepřítomnosti přepážky mezi touto komůrkou a plodovou buňkou. Pryskyřičná přepážka se vyskytovala u hnízd druhů *R. septemdentatum* a *R. infuscatum*, u zbylých dvou druhů chyběla. Druhý zásadní rozdíl byl také v tom, zda vstupní komůrka byla, či nebyla vyplňovaná minerálním nebo rostlinným materiálem. Řídce vyplněné byly tyto komůrky u hnízd druhů *R. septemdentatum* a *R. sticticum*, zatímco zbylé dva druhy ponechávaly tyto prostory prázdné.

V rámci výzkumů jsme také přímo pozorovali hnízdící samice u druhů *R. septemdentatum* a *R. sticticum*. Oba druhy ke stavbě hnízda využívaly ulity ukryté pod kameny nebo ve štěrbinách kamenných zdí a dále s nimi nijak nemanipulovaly. To je rozdíl oproti pozorování druhu *R. siculum*, které provedli Erbar & Leins (2017), kdy hnízdící samice zahnízděné ulity zahrabávaly do písku.

U třech druhů (*R. septemdentatum, R. sticticum* a *R. siculum*) jsme potvrdili přezimování ve stádiu dospělce. Naše pylové analýzy ukázaly, že druhy rodu *Rhodanthidium* jsou polylektické, ale také naznačily preferenci druhu *R. sticticum* na rostliny čeledi Fabaceae.

V hnízdech jsme nalezli velmi málo parazitů, pouze u druhu *R. sticticum* byla dvě hnízda parazitována zlatěnkou *Chrysura refulgens* a několik hnízd bylo napadeno pylovými roztoči *Chaetodactylus* cf. *anthidii*.

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# 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

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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* Lepeletier (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; Kasparek 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; Kasparek 2019). The nesting biology of the only *Meganthidium* species (*R. superbum* (Radoszkowski)), which is distributed in Turkey and the Middle East, remains unknown (Kasparek 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 (Kasparek 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 (Kasparek 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; Kasparek 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.

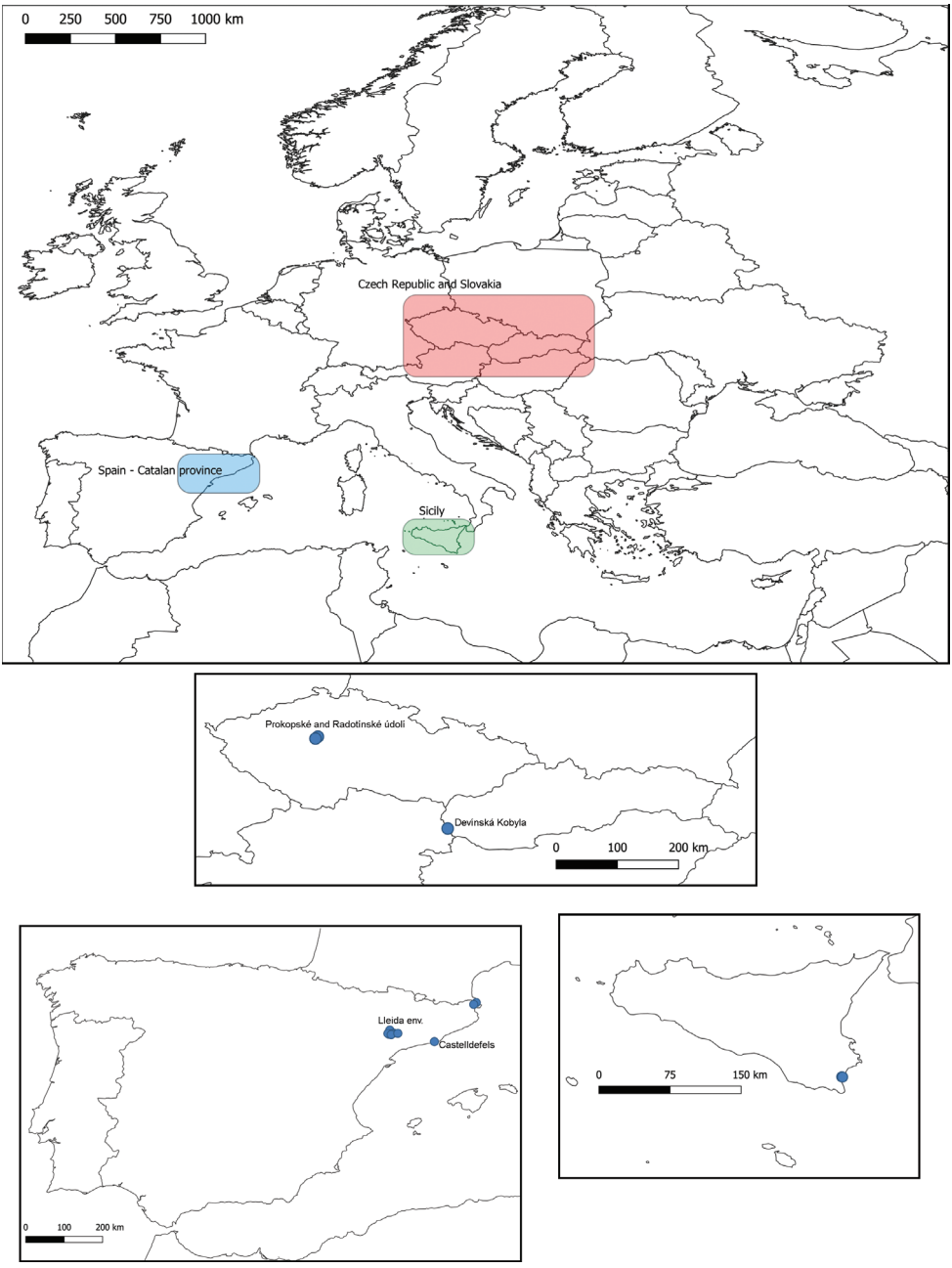


**Figure 1.** Location of study sites (blue dots) in the three regions surveyed.

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 ($H_2SO_4$) and acetic anhydride $(CH_3CO)_2O$ 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

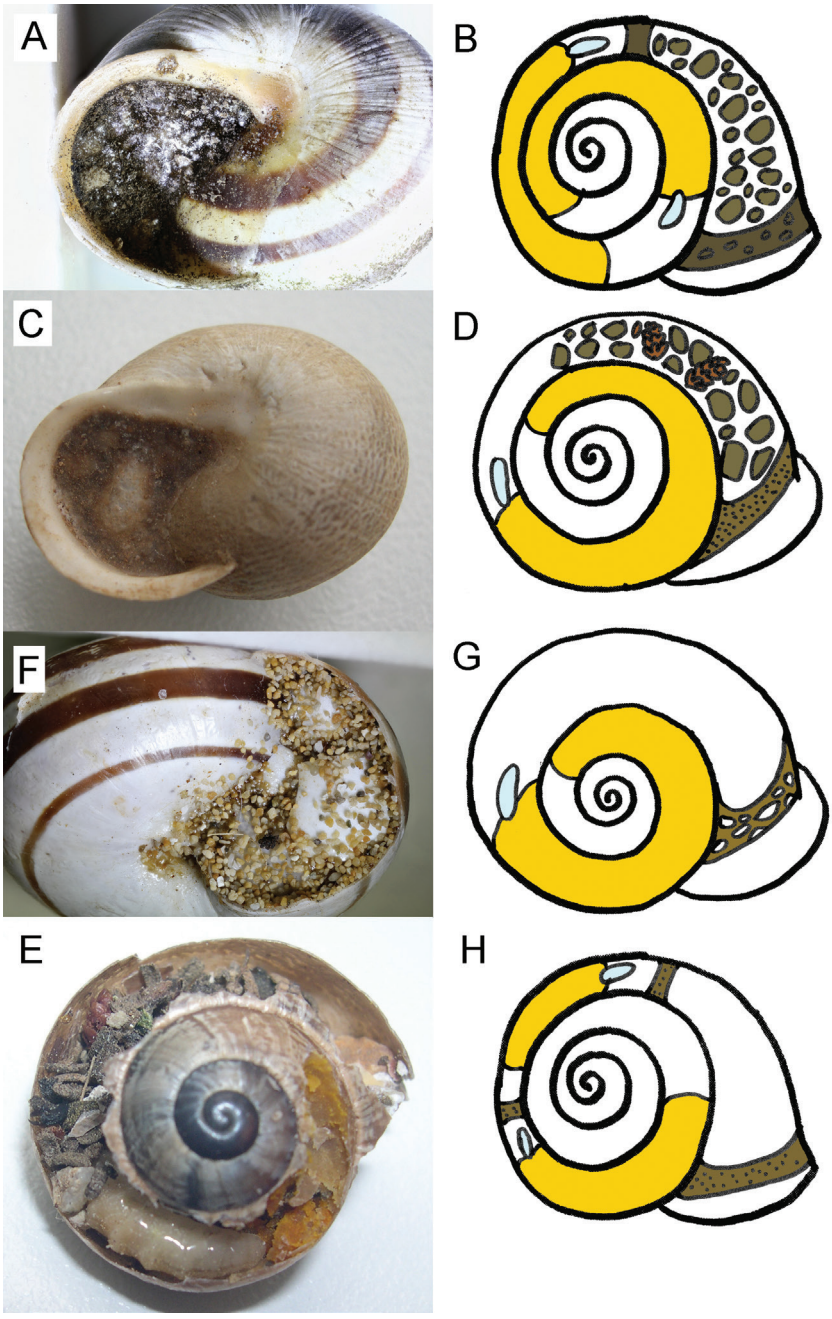


**Figure 2.** Photos and schematic drawings of nests of four species of *Rhodanthidium*. *Rhodanthidium septemdentatum* **A** shell of *Caucasotachea vindobonensis* with closing plug made of resin **B** schematic drawing of the inner nest structure in the shell. *Rhodanthidium sticticum* **C** shell 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.

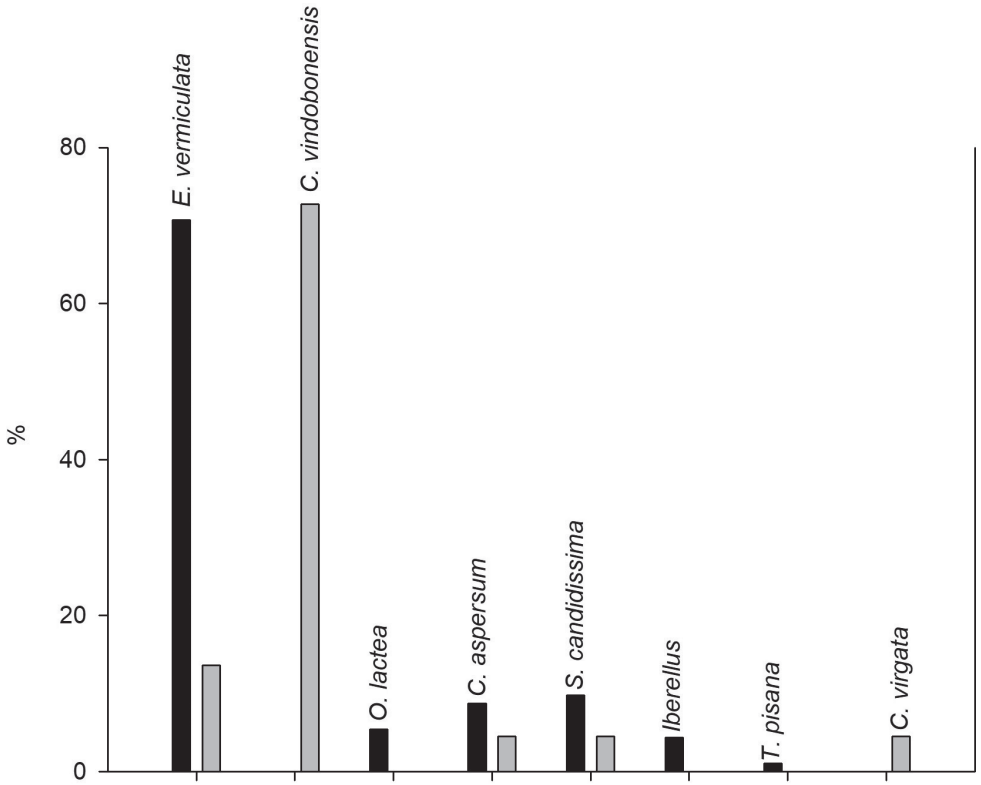


**Figure 3.** Proportions (in %) of shells used by *Rhodanthidium sticticum* (black columns) and *Rhodanthidium septemdentatum* (grey columns).

(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), *Cernuella 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

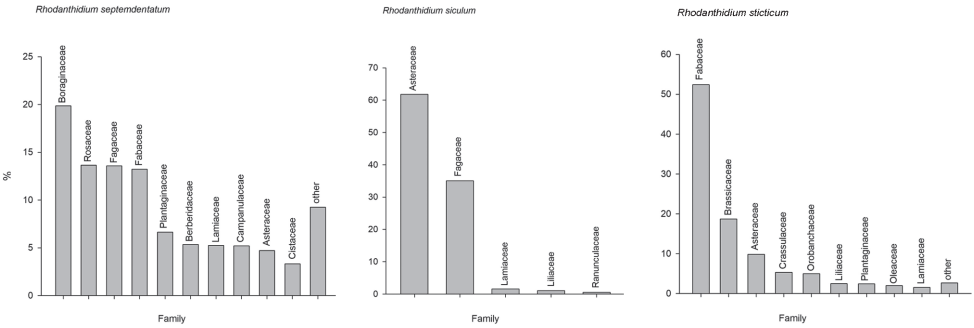


**Figure 4.** Proportions (in %) of pollen grains of plant families in studied nests of three species of *Rhodanthidium*.

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; Kasparek 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 | *R. infuscatum* | *R. septemdentatum* | *R. siculum* | *R. sticticum* |
|---|---|---|---|---|
| **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. rufohirta* 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; Kasparek 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; Kasparek 2019). Previous observations have indicated that *R. septemdentatum* females collect pollen for their brood primarily from the Fabaceae and Lamiaceae families (Kasparek 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; Kasparek 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; Kasparek 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 (Kasparek 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 (Kasparek 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 1. 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, Castelldans, 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 km$^2$ 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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## 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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