



Lack of evidence for chemical integration of the cuckoo-bee *Stelis nasuta* (Latreille, 1809) and *Coelioxys aurolimbata* (Förster, 1853) with their main host *Megachile parietina* (Geoffroy, 1785)

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Abstract – *Megachile parietina* is a solitary megachilid species, which sometimes nests in large aggregations. Such a condition attracts a diverse entomological fauna, including several parasites. Here, we focused on two cuckoo-bees of this species, *Stelis nasuta* and *Coelioxys aurolimbata* (Megachilidae) and investigated if chemical integration with the host favours their reproductive success. Lipids on the epicuticle and in the Dufour's gland secretion, possibly contributing to protecting the eggs against dehydration, of the three species showed species-specific mixtures of long-chained linear alkanes and alkenes. Moreover, contrary to what has been reported for some cuckoo-bees of the genus *Nomada*, we found no evidence that the mandibular glands of parasite males contribute to female chemical mimicry. Therefore, we found no indication that chemical integration is part of the adaptive strategies of these two brood parasites.

cuticular hydrocarbons / Dufour's gland / mandibular glands / ovaries / parasite adaptations

1. INTRODUCTION

Among Anthophila, brood parasite species exploiting nests and the food storage of other bees, are present in four out of seven families, namely Apidae, Colletidae, Halictidae and Megachilidae (Danforth et al. 2019). Most of these parasites

exploit solitary species, although parasites of social bees are found in Apidae (subfamilies Xylocopinae and Apinae) and Halictidae (subfamily Halictinae). Since obligatory brood parasite (cuckoo) bees neither build a nest nor collect pollen for their brood, they have lost structures linked to those tasks and some have an armoured exoskeleton, protecting them against host attacks (Michener 2000; Danforth et al. 2019).

Relatively little is known about the behaviour and adaptations of parasitic bees. Since brood parasitism reduces the host's reproductive success, hosts are expected to defend their nests, while parasites are expected to limit interference with

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their hosts and maximize their fitness by laying a considerable number of eggs (Polidori et al. 2020). Indeed, some remarkable differences between female cuckoo bees and pollen-collecting bees have been reported for the reproductive system (Iwata and Sakagami 1966). Generally, pollen-collecting bees lay one egg per day, and their ovaries, each formed by three to four ovarioles, mature only one egg at a time (Iwata and Sakagami 1966). On the contrary, cuckoo bees may have a higher number of ovarioles and multiple mature eggs at once, since, if the opportunity arises, they can lay several eggs per day (Iwata and Sakagami 1966).

Long ligula bees (Apidae and Megachilidae) encompass most of the cuckoo bee species which are divided into three broad categories based on the parasitic strategy: “adult, closed-cell parasitism” (AC), “larval, closed-cell parasitism” (LC) and “larval, open-cell parasitism” (LO) (Litman et al. 2013). AC parasites, such as some species of the *Stelis* clade, lay their eggs in cells that are already closed and supplied with pollen. Females open a hole in the cell, kill the host egg or larva and lay their egg, which is usually larger than that of the host. LC parasites, as some species of the tribe Dioxyni, lay their eggs in closed cells and their larvae kill the host brood and also other parasitic larvae (Litman 2019). LO parasites comprise most cuckoo bees. Females lay one or more eggs in an open cell not yet fully supplied with pollen. These latter parasites need to deceive the hosts from detecting both their visits to the nests and the presence of their brood (Litman 2019). The eggs of LO parasites are usually smaller than those of their hosts and are often hidden in the cell wall (Rozen 2003). Host females, particularly those forming nest aggregations, may perform a very active defence against these parasites (Litman 2019). LO parasites often linger close to the host nests waiting to inspect the cells or to lay eggs as soon as the host female leaves (Litman 2019).

In addition to behavioural strategies, chemical strategies have also been proposed for LO parasites (Litman 2019) and are expected especially for parasites of social hosts, where nests are unlikely to be unguarded. In those cases,

the host-parasite arms race could have led LO parasites to develop chemical strategies similar to those described for the social parasites of the *Psithyrus* subgenus (Ayasse and Jarau 2014). Chemical integration of brood parasites into the host nests can be achieved through three different strategies (Dettner and Liepert 1994): (i) chemical mimicry when recognition pheromones are produced *ex novo* by the parasite and cause it to be recognized as conspecific or nestmates by the host; (ii) chemical insignificance when the parasite reduces recognition cues; (iii) chemical camouflage when the parasite acquires chemicals that lead it to match with its host, as often found in social parasites.

Epicuticular lipids, containing complex mixtures of long chain hydrocarbons, the main recognition pheromones in insects (Bagnères and Blomquist 2010), have been studied for parasites of social insects, including bees, while limited information is available for brood parasites of solitary bees. A resemblance between the alkene isomer profiles has been found for host-parasite couples of the genus *Bombus* (Martin et al. 2010). Within the large parasitic genus *Sphecodes* (Halictidae: Halictinae), *S. monilicornis* appears to use chemical insignificance to parasitise its *Lasioglossum* social hosts, while *S. puncticeps* does not seem to integrate chemically in the host colonies in any way (Polidori et al. 2020). In hymenopterans, part of epicuticular lipids is produced or absorbed from the haemolymph by accessory exocrine glands such as the thoracic and the abdominal Dufour’s glands (Blomquist and Bagnères 2010). In bees, the main components of Dufour’s gland secretion are long-chained hydrocarbons, oxygenated compounds such as aliphatic esters and macrocyclic lactones, and some more volatile compounds (Hefetz 1987). Since this gland is associated with the reproductive system, its lipidic secretion may contribute to protecting the eggs against dehydration. In several species, and especially in the ground-nesting ones, it contributes to waterproofing the cells (Hefetz 1987). Pheromonal functions have also been reported; in the honeybee, it was suggested that the secretion contains a queen-specific egg-marking pheromone

(Katzav-Gozansky et al. 2004), while a nest-marking function has been reported for solitary bees (Hefetz 1987; Fischman et al. 2017). The Dufour's gland secretion has been analysed for several solitary bees (Hefetz 1987), but only in a very few parasites (Tengö et al. 1992). Therefore, no information is available about its possible role in the chemical integration of the parasites, and if its secretion helps to protect eggs from the destruction by the host either by contributing to their mimicry or through semiochemicals affecting the host's behaviour. For a few species, it has been reported that the gland has a small size (Tengö and Bergström 1977; Danforth et al. 2019). Interestingly, Tengö and Bergström (1977) reported that the main volatile component of the Dufour's gland of some *Andrena* and *Melitta* species corresponds to that found in the mandibular glands of the males of their *Nomada* parasitic species. The authors suggested that *Nomada* males "perfume" the females with these compounds during mating and this helps reduce aggression by the host. Among brood parasites, the mandibular gland secretion has also been studied in some *Coelioxys* and *Epeolus* species (Tengö et al. 1982), and in *Holcopasites* (Hefetz et al. 1982), but such a function has not been demonstrated.

This work focuses on three megachilid species, the brood parasites *Coelioxys aurolimbata* and *Stelis nasuta* and their common main host, the solitary but often gregarious species, *Megachile* (subgen. *Chalichodoma*) *parietina*. Like all the *Chalichodoma* species, *M. parietina* builds very hard and durable nests by mixing soil and pebbles with cephalic secretions. Both parasites belong to two exclusively parasitic clades and perform the LO-type strategy, although cell opening by the *Stelis nasuta* has also been reported (Amiet et al. 2004). Limited information is available about their reproductive biology and parasitic strategies. Larvae of *Coelioxys* are likely to kill the host larvae and possibly even conspecific larvae with their sharpened mandibles (Rozen 1967). On the contrary, based on the shape of the mandibles, Rozen (1967) hypothesised *S. nasuta* larvae not to be hospicidal and this could be an adaptation to limit fratricidal

cannibalism since several larvae can develop within the same cell (Kasperek 2015).

Although both species have two or three host species (*M. parietina* and *M. ericetorum* for *C. aurolimbata* and three species of the subgenus *Chalichodoma* for *S. nasuta*), in the study site *M. parietina* is the only host for *S. nasuta* and the most abundant for *C. aurolimbata* (Monterastelli et al. in press). Since we observed that *M. parietina* does not seem to inspect the cells more intensively after they are visited by the parasites, we wonder if a form of chemical integration, as defined above according to Dettner and Liepert (1994), could favour the reproductive success of the parasites. Thus, we studied the epicuticular lipids and the secretion of the Dufour's and the mandibular glands of the three species. Moreover, we investigated oocyte maturation.

2. MATERIALS AND METHODS

2.1. Site description, observation of insect behaviour and collection of specimens

Fieldwork was carried out in the late spring (May–June) of 2018, 2019, 2020 and 2021 near Montespertoli (FI, Italy, 43°38'50.8" N 11°01'09.8" E) in a private estate where a large nest aggregation of the host bee, *M. parietina* (about 600 females), is found on the vertical surface of a barn beam. Fieldwork was performed at about 7-day intervals in 2018, 2020 and 2021 and about 3 or 4-day intervals in 2019. Both observations and insect collection were performed from about 9:30 to 13:30.

In all 4 years of fieldwork, we observed that the flight period of *S. nasuta* preceded that of *C. aurolimbata*. To evaluate the relative presence of the two parasites and their sex, in 2018 and 2019, we captured in each field section a limited number of specimens, trying to respect the proportion between the observed specimens of the two species (while sexes are not easily recognized in flying insects). Specimens were captured using an entomological net and individually placed in 15 mL Falcon tubes. In total, for the following

analyses, we captured 19 females of *M. parietina*, 13 *C. aurolimbata* and 16 *S. nasuta*. Moreover, we captured 13 and 5 males respectively of *C. aurolimbata* and *S. nasuta*. Samples were stored in a field cooler and then frozen at -20°C until they were analysed.

2.2. Dissections and measurements

A trinocular stereoscope (ZEISS Stemi 508) equipped with a video camera (Axiocam 105 Color) was used for dissections, measurements and pictures. The reproductive system was dissected by using entomological tweezers, and for each female, ovarioles and mature oocytes were counted. Moreover, the length (E =egg length) and the width of the largest oocytes, the width of the mesosoma (as the distance between the outer extremities of the tegulae, M =mesosomal width), and the length of the Dufour's gland were measured using the Zen lite program.

Dufour's glands were then excised and transferred into a vial containing a 200 μl glass conical insert.

According to Iwata and Sakagami (1966), the egg index was calculated as the ratio between (E) and (M) and compared between species by one-way ANOVA. In addition, we considered the egg size classes as defined by the same authors: *dwarf* ($E/M \leq 0.50$); *small* ($0.50 < E/M \leq 0.75$); *medium* ($0.75 < E/M \leq 1.00$); *large* ($1.00 < E/M \leq 1.10$); *giant* ($1.10 < E/M$). The projection of the largest egg onto the plane was also calculated as a proxy of the egg's surface.

Mandibular glands of males and females of the three species ($N=3$ for females of *M. parietina*; $N=4$ and $N=10$ for females and males of *C. aurolimbata*; $N=3$ and $N=5$ for females and males of *S. nasuta*) were carried out using a scalpel to cut the head at the level of the clypeus. The lower part was inserted in a vial containing a conical insert and stored at -20°C .

2.3. Chemical analyses, compound identification and data analysis

Chemical analyses of cuticular lipids were carried out on 10 females of *M. parietina*, 13 *C. aurolimbata* and 19 *S. nasuta*. Cuticular lipids

were extracted as follows: single specimens were placed in a 2 mL vial with the head facing downwards and added with pentane to almost cover the insect completely (respectively 800 μL , 400 μL and 400 μL for each species) but not the extremity of the abdomen which had previously been dissected. The extraction was made at room temperature for 5 min; extracts were then evaporated under a gentle nitrogen stream and then resuspended in 100 μL of heptane.

Three microliter of the solution were injected on a GC-MS 7820 GC system-5977B MSD (single quadrupole, Agilent Technologies) installed with a 19091S-433UI column (stationary phase, 95% PDMS, 5% benzene; 30 $\text{m} \times 0.25$ mm, Agilent Technologies) and equipped with a 7693 autosampler (Agilent Technologies). Helium (1 mL/min) was used as carrier gas. The oven temperature program was set as follows: 70°C (3 min); $15^{\circ}\text{C min}^{-1}$ up to 150°C (3 min); $5^{\circ}\text{C min}^{-1}$ up to 320°C (17 min). Electronic ionization was carried out at 70 eV and m/z values were acquired in the range of 50–650. A mixture of linear alkanes (C_{21} – C_{43}) was injected under the same analytical conditions to calculate the retention indexes of the target analytes. The GC-MS traces of the three species were carefully aligned and compared with that obtained for the cuticular lipid extracts of *Apis mellifera* for which the alkene isomers are well described (Dani et al. 2004). Data were analysed using the software Agilent MassHunter Qualitative Analysis B.07.00. Compounds were identified by spectra comparison with Wiley275 and NIST11 libraries, as well as by comparing their retention indexes with those reported in spectral libraries and, for cuticular hydrocarbons through the manual interpretation of spectra. The relative abundance of each compound was calculated as the percentage of the area underlying the total ion chromatogram (TIC) of each peak to the sum of the area of all the peaks, excluding contaminants and free fatty acids, which are ubiquitous in insect tissues (Dani et al. 2003). A multi-dimensional scaling analysis was performed on the whole specimen dataset (SPSS, PROXSCAL); city block distances, which reduce outlier emphasis, were calculated.

Headspace analyses have been carried out on 6 Dufour's glands of *M. parietina*, 7 of *C. aurolimbata* and 14 of *S. nasuta* by absorbing the volatiles from the sealed vials containing the excised glands with an SPME fibre (Divinylbenzene, Carboxen, Polydimethylsiloxane, DVB/CAR/PDMS; 1 cm, 100 μm thickness) at 60 °C for 10 min. Fibres were desorbed for 10 min in the injection port of the same equipment described above, under the same conditions except for the temperatures, which were the following: 45 °C (2 min); 10 °C min^{-1} up to 200 °C (3 min); 15 °C min^{-1} up to 300 °C (2 min). A mixture of linear alkanes (C_{15} – C_{30}) was injected and used as references to calculate the retention indexes of the sample analytes. After the SPME extraction, we added 15 μL of heptane into the same vial and vortexed it to recover the semi-volatile compounds. Analyses were carried out using the same protocol as epicuticular lipids. To evaluate if parasite adaptation is based on chemical insignificance, for each specimen, we divided the amount of cuticular lipids (as resulting from the sum of the peak areas in the body extracts) by the square of the mesosome width and compared these values between species (one-way ANOVA). Similarly, we evaluate the insignificance of eggs by considering the amount of hydrocarbons in the solvent extracts of the Dufour's gland divided by the oocyte surface.

SPME sampling and analysis of mandibular gland samples were performed like for Dufour's glands.

3. RESULTS

3.1. Parasite presence and behaviour observation

Figure 1 reports the proportion of the two cuckoo-bee species in May and June as estimated by the number of captured specimens in 2018 and 2019. In both years, we found more *S. nasuta* females and males active in May and more *C. aurolimbata* active in June. Both males and females of the two species were present at the nesting site, but each year we observed that

the ratio M/F was lower for *S. nasuta* than for *C. aurolimbata*. Many males of this latter species were observed to fly around a quite limited area above the shelter where the nesting site was located, where probably courtship occurred.

During these months, many females of both parasites fly constantly in front of the cells, sometimes very close to the host females. Both parasite species fly very fast, especially *C. aurolimbata*, and visit very quickly the nests while the host females are foraging. Moreover, while *S. nasuta* females often sit very close to the nests occupied by hosts, *C. aurolimbata* females tend to fly all the time. Despite the presence of a few tens of host females active on the nesting site (about 70 at the end of May 2019, Monterastelli et al. [in press](#)), and the high number of active parasites, we only observed a host female chasing a parasite on a couple of occasions. By observing hosts returning to cells just visited by parasites, we never saw them removing eggs or other material from the cells and no peculiar behaviours were noticed. This contrasts with the long inspection we observed after a conspecific *M. parietina* female enters a cell already cared for by a resident female.

3.2. Ovaries and ovary index

All three species present three ovarioles per ovary, as typical of the family Megachilidae (Figure 2A). However, while *M. parietina* ovaries have normally only one large oocyte and others of much smaller size, all parasite ovaries contained at least two large oocytes and several others of similar size. Figure 2B shows the egg size for the three species (measured in 9 *M. parietina*, 11 *C. aurolimbata* and 14 *S. nasuta* females). The higher variability observed in the hosts could be due to specimens which had laid an egg very recently and whose oocytes were still developing. In the case of *M. parietina* re-adapting an old cell or constructing a new one, providing it with pollen and finally closing it requires normally 3–6 days (Dani, unpubl. data). Therefore, egg development can be slower than in other solitary bees. On the contrary, brood

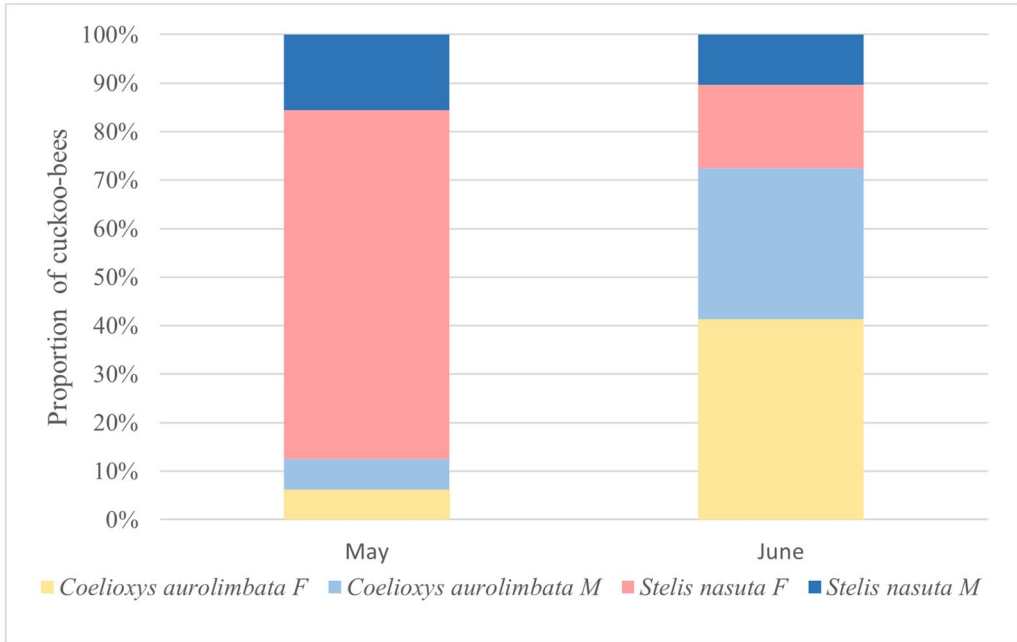


Figure 1. Female and male proportions of the two cuckoo-bee species, active in the study area in May and June; total data for years 2018 and 2019.

parasites may lay several eggs per day during a relatively short flight time, especially within large aggregations of host nests. Figure 2C shows the egg index (Iwata and Sakagami 1966). By considering the average values, the eggs of the two parasites are classified as “dwarf”, while those of the host are as “small”.

3.3. Dufour’s gland size

Considering the hypothesis that Dufour’s gland secretion could be involved in the chemical integration of the parasite eggs into the host nests, we analysed both the size of the gland and the volatile and semi-volatile constituents of its secretion. Due to its small size, the gland could not be successfully dissected in all the specimens. As expected, the size of the gland, as estimated from its length, varied remarkably according to the size of the species, with *S. nasuta* having much smaller glands than the other two species ($N=6$, $2734.6 \pm 697.2 \mu\text{m}$; $N=6$, $2054.7 \pm 127.7 \mu\text{m}$;

$N=2$, $666.1 \pm 45.3 \mu\text{m}$, respectively, for *M. parietina*, *C. aurolimbata* and *S. nasuta*). By considering the gland length divided by the mesosoma width, *C. aurolimbata* showed a higher value, but not statistically significant (Student’s *t*-test), than the host (0.59 ± 0.17 ; 0.61 ± 0.04 ; 0.28 ± 0.02 , respectively, for *M. parietina*, *C. aurolimbata* and *S. nasuta*).

3.4. Epicuticular lipids and Dufour’s gland secretion

The epicuticular extracts and the Dufour’s gland secretion of the three species contain linear alkanes and alkenes ranging from 17 to 33 carbon atoms (Figure 3; Table S1). In each species, most compounds are present in both samples, but a higher concentration of longer-chained alkenes is found in the epicuticular compounds. Although several components were in common between the three species, some were exclusive, such as C_{23} and C_{25} monoenes present

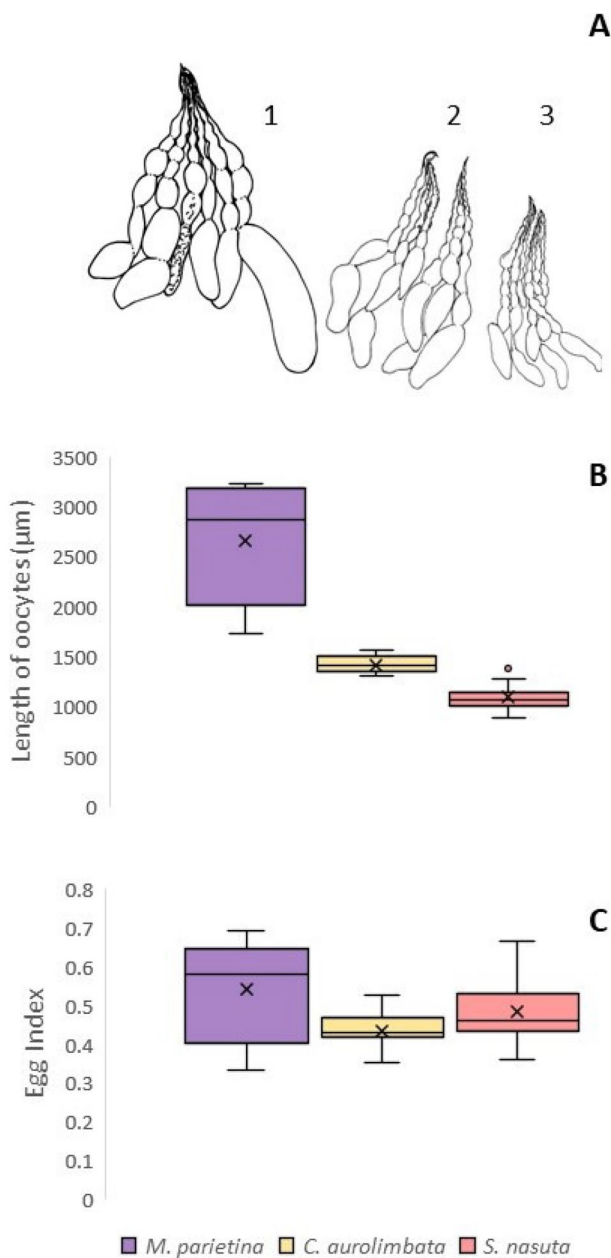


Figure 2. **A** Ovarian structure of (1) *M. parietina*, (2) *C. aurolimbata* and (3) *S. nasuta*. **B** Boxplots of the length of the most developed oocytes measured (*M.p* $N=16$; *C.a* $N=11$; *S.n* $N=13$). **C** Boxplots of the egg index calculated according to Iwata and Sakagami (1966) in the three species. X indicates the average value (one-way ANOVA, $fd=33$; $F=3.389$; $P=0.047$).

in high percentages in the cuticular extracts of *C. aurolimbata*, and a C_{33} diene in *S. nasuta*. A clear species-specificity of the cuticular lipids

and Dufour's gland secretion is evident both from the profile (Figure 3) and from the MDS analysis (Figure S1).

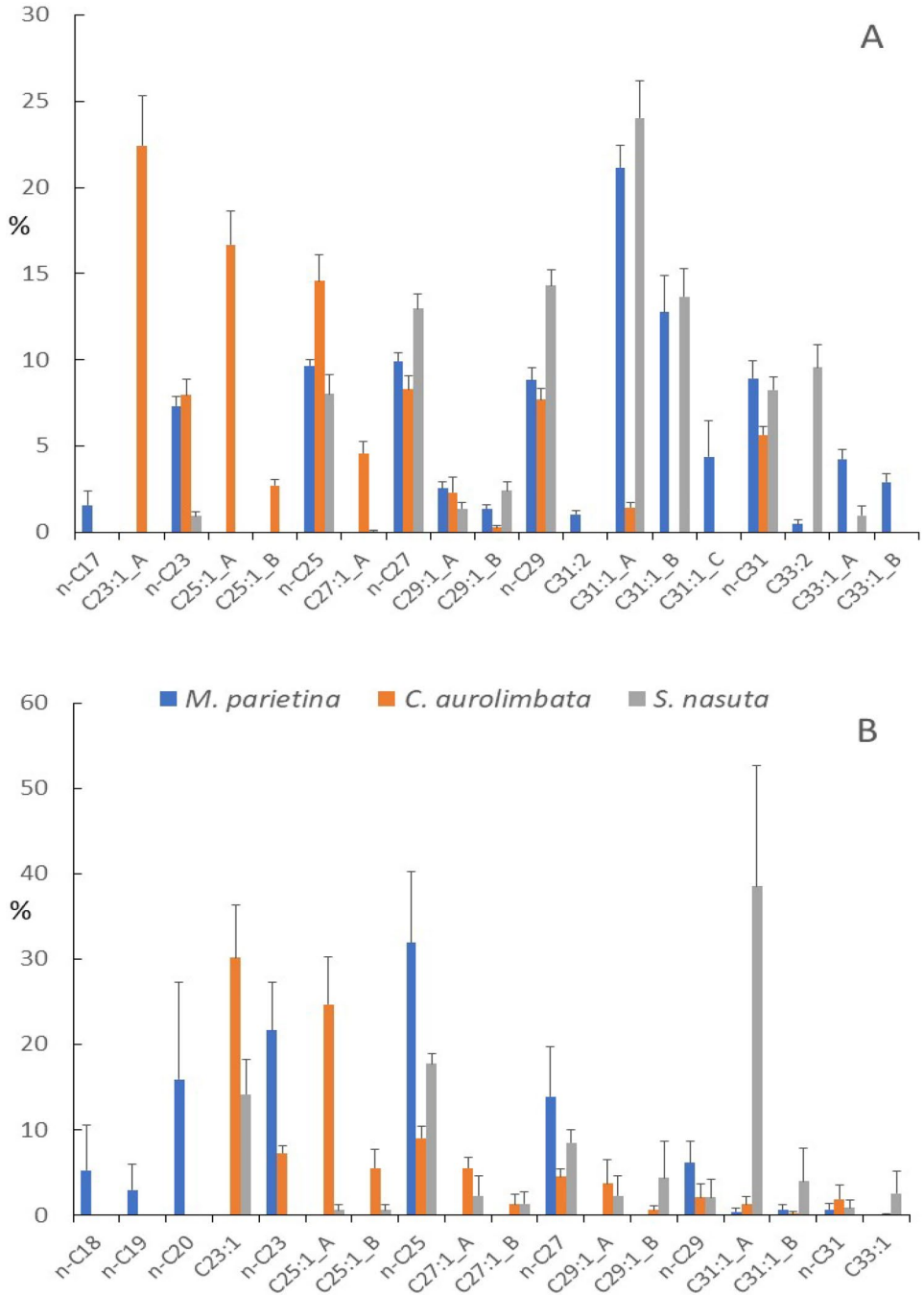


Figure 3. Average percentage (and ES) of hydrocarbon components in the epicuticular lipids **A** and Dufour's gland **B** of the three studied species. Epicuticular lipids and Dufour's gland secretion were analysed respectively for 10 and 5 *M.p.*, 13 and 6 *C.a* and 19 and 2 *S.n.*

The comparison between the total amount of cuticular lipids extracted from each specimen corrected by the square of the mesosome width (Figure 4A), did not show a difference among the three species (ANOVA, $F = 2.792$, $fd = 28$; NS). Similarly, the amount of hydrocarbons per egg surface was not lower for the parasite females, on the contrary, we observed that consistently with the gland size, this value was higher for *C. aurolimbata* females (Figure 4B), although not significantly (ANOVA, $F = 1.207$, $fd = 12$; NS).

The more volatile constituents of the gland secretion were also analysed. The gland of the host does not show additional compounds than those already identified. The same was found for the gland secretions of *C. aurolimbata*, except for two spiroacetals found only in one sample and therefore not shown in Table S1. These same compounds were found in the

glandular secretion of the mandibular glands (Table S3). On the contrary, some acetates of aliphatic alcohols (Table S3) were found in the glandular secretions of *S. nasuta* in addition to a few aliphatic hydrocarbons already identified.

3.5. Mandibular gland secretion

No compounds were identified in the samples of both sexes of *M. parietina* and *S. nasuta*, suggesting that these glands were not active at the time of sampling. In contrast, a quite complex mixture was found in the mandibular secretions of *C. aurolimbata* (Table S3). Most compounds correspond to those already reported for *C. quadridentata* and *C. mandibularis* (Tengö et al. 1982), i.e., aliphatic short-chained secondary alcohols, 3-ketones, aldehydes and two

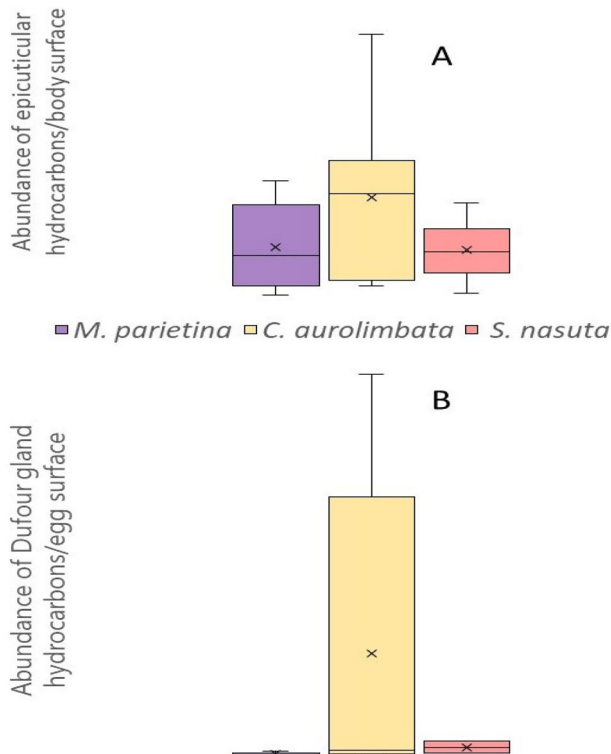


Figure 4. **A** Epicuticular hydrocarbon abundance corrected by a proxy of the body surface in *M. parietina* ($N=7$), *C. aurolimbata* ($N=12$) and *S. nasuta* ($N=11$). **B** Lipid abundance in the Dufour's gland divided by egg area (*M.p* $N=5$; *C.a* $N=6$; *S.n* $N=2$).

spiroacetals. Moreover, based on the retention time and the molecular ions, we hypothesized the presence of an unsaturated spiroacetal, whose molecular formula is $C_{10}H_{16}O_2$. The MS spectrum of the compounds is reported in Figure S1.

4. DISCUSSION AND CONCLUSIONS

Both in *C. aurolimbata* and *S. nasuta* each ovary contains, at the same time, several mature oocytes. This is consistent with the biology of larval, open-cell cuckoo-bees that can parasite several cells per day. *M. parietina*, on the other hand, shows only one mature or almost mature oocyte at each time. According to the class indexes by Iwata and Sakagami (1966), the oocytes of both parasites fall, as for many other brood parasites, into the “dwarf” category while those of the host in the “small” class. Therefore, oocyte development in the two parasites has the same features reported for other larval, open-cell parasites.

Cuticular lipids have been studied in only two other species of Megachilidae, *Osmia lignaria* and *M. rotundata* by Buckner and coworkers (2009). Similarly, to what was reported by these authors, the compounds identified in the three species we studied are mainly alkanes and monoenes in the range C_{23} – C_{33} . These same compounds are the major components of cuticular lipids in all the Anthophila species studied so far (Pherobase.com). With the only exception of traces of methyl heptacosane found in *S. nasuta*, we did not identify methyl alkanes, which in other bees, including *O. lignaria* and *M. rotundata* (Buckner et al. 2009), are minor components. With regard to oxygenated compounds, we only identified an aliphatic aldehyde in *S. nasuta*.

The Dufour’s gland secretion contained the same compounds as identified in the cuticular hydrocarbons, and some acetates of fatty alcohols in *S. nasuta*. Waxy esters, in low concentrations, have been reported for *O. lignaria* and *M. rotundata* (Pitts-Singer et al. 2017), but not here. Therefore, based on the information so far available (Pherobase.com), the Dufour’s gland secretion of Megachilidae seems to contain less oxygenated compounds when compared to other

lineages of solitary bees, such as Halictidae and Colletidae, where several macrolactones are present, and Andrenidae for which several long-chained terpenoids are known.

Despite containing the same compounds, the epicuticular lipids of the three species are remarkably species-specific and the amount of lipids on the body surface of the parasites was not lower than on the host. In studies on social insects, alkenes and methyl-branched alkanes have been found to be more relevant as nestmate recognition cues than alkanes (Dani et al. 2001; 2005) and the profile of alkene positional isomer to be relevant for the integration of some parasitic *Bombus* species in the host colonies (Martin et al 2010). Both the parasitic species we studied were dissimilar from the host for the presence of alkenes, with *C. aurolimbata* having four monoenes ($C_{23:1}$ – $C_{27:1}$) not present in the host and *S. nasuta* showing a different pattern of C_{33} alkenes. Therefore, based on these results, the hypothesis that chemical mimicry or chemical insignificance is part of the parasite adaptation was not confirmed and cannot explain why, as we observed in the field, *M. parietina* does not react to parasites approaching their nests and does not perform peculiar behaviours when returning to cells visited by parasites.

Danforth et al. (2019) report that the Dufour’s gland of kleptoparasite species is greatly reduced as they do not have to build and waterproof the nest; on the contrary, the same gland is enlarged in some parasitic *Bombus* where it produces some allomone substances protecting the parasite from the host (Lhomme and Hines 2018). The Dufour’s gland size of the parasitic species we studied, corrected by their body size, was found to be smaller than the host in *S. nasuta*, but not in *C. aurolimbata*, and similarly to cuticular hydrocarbons, the secretion profiles are different between species. Therefore, if the Dufour’s gland secretion participates in the production of the hydrophobic layer of the eggs (see introduction), its secretion does not seem to favour egg chemical mimicry.

Tengö and Bergström (1977) suggested that males of some species of the brood parasitic genus *Nomada* help females to parasitize *Andrena*

and *Melitta* nests by transferring their mandibular gland secretion which contains the main components of the host Dufour's gland during mating. In our analyses, we only found volatiles and semi-volatile compounds in the mandibular glands of *C. auro limbata* and the compounds present were mostly the same in both sexes, as already reported for other *Coelioxys* species (Tengö et al. 1982). Except for a few linear hydrocarbons, none of the present compounds has been identified in any sample of the host species. Remarkable differences between the cephalic secretions have also been reported for the kleptoparasite *Holcopasites calliopsidis* and its host *Calliopsis andreniformis* (Hefetz et al. 1982).

Therefore, none of our results supports the hypothesis that chemical integration through either mimicry or insignificance, occurs in these two parasitic species neither at the adult nor at the egg stage. Since these parasite females do not conceal themselves when flying in search of suitable cells nor chemically integrate with the hosts, the reason for the apparent lack of attention by the hosts remains enigmatic. One possible defence by the host could be the active search and destruction of the parasite eggs inside the cells, but the irregular structure and stony texture of nests make it difficult to observe in the field the behaviour inside the cells.

So far, chemical integration with the hosts has only been reported for parasites of social species (Ayasse and Jarau 2014; Polidori et al. 2020) for which facing the host is inevitable. Given the limited information on cuckoo-bees' chemoecology, further studies considering different bee lineages are needed to investigate if this adaptation also occurs in parasitic bees of solitary species.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1007/s13592-023-01031-x>.

AUTHOR CONTRIBUTION

FRD conceived the work. All authors contributed to the field work. MM, OCM, EZ and FRD performed the analyses. MM, OCM and FRD wrote the original draft. All authors read and approved the final manuscript.

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DATA AVAILABILITY

All data generated or analysed during this study are included in this published article and its supplementary information files.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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