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## Nests, Floral Preferences, and Immatures of the Bee *Haetosmia vechti* (Hymenoptera: Megachilidae: Osmiini)

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### ABSTRACT

Herein we describe the nests (including structure, closure, orientation, and depth of cells) of the bee *Haetosmia vechti* Peters found nesting in Rehovot, Israel. The nesting biology of *H. vechti* mirrors the ancestral nesting biology within the *Osmia* group of the Osmiini. Nests in sandy soil consist of an excavated burrow, ending below in a small cluster of vertical cells. The cells possess firm walls of masticated leaf pulp of *Centaurea procurrens* Spreng. and *Heliotropium suaveolens* M. Bieb., and are covered with pebbles and sand grains.

The last larval instar and pupa of *Haetosmia vechti* are described, as is its cocoon. The immature stages exhibit the basic features of megachilid bees, but tend to have a thinner body vestiture compared to other studied taxa.

In addition, we report new information on and review published accounts concerning the pollen collecting behavior of the genus *Haetosmia* Popov, which contains three species. Pollen

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taken from scopal hairs of 68 females collected at 17 sites in Turkestan, Morocco, Israel, and the United Arab Emirates was identified as originating solely from *Heliotropium* L. (Boraginaceae), which strongly suggests that all three *Haetosmia* species are narrowly oligolectic on this plant genus. In females of all three species, the second segment of the labial palpus is densely covered with rather long, apically curved and capitate bristles, an adaptation to collect *Heliotropium* pollen from anthers that are hidden inside the narrow corolla tube. Similar pollen-harvesting bristles specifically adapted to exploit flowers of *Heliotropium* seem to have evolved independently a number of times on different continents, in bees of four families.

## INTRODUCTION

The genus *Haetosmia* Popov, 1952 (Megachilidae: Osmiini), consists of three species of small, robust, hairy bees distributed in the western Palearctic region. *Haetosmia* is a member of the *Osmia* group of genera within the tribe Osmiini (Praz et al., 2008). Most phylogenetic analyses placed it as the basal branch within this *Osmia* group (Praz et al., 2008; Sedivy et al., 2013a), although in some analyses it was the sister group to a large clade consisting of *Wainia*, *Osmia*, *Atoposmia*, and *Ashmeadiella* (Praz et al., 2008). In contrast to the *Heriades* group, which consistently uses resin as nesting material, the *Osmia* group uses a diverse variety of nesting material. Knowledge of the nesting biology and phylogenetic position of *Haetosmia* is thus important for elucidating the evolution of nesting behaviors within the Osmiini.

Since the nests of *Haetosmia* were hitherto unknown, our discovery of nests permits us to describe for the first time the nesting biology and immature stages of one of the species, namely *Haetosmia vechti* Peters, 1974. We also provide new data and summarize the known floral preferences and pollen collecting behavior of the genus. Field research was carried out by A.G. and G.P. J.G.R. described the cells, cocoons, and immature stages collected, and C.P. and G.R. performed the chemical analysis of cell walls. A.M. and C.S. provided data on floral preferences and pollen collecting behavior for the genus. A.G., A.M., C.P., G.P., and J.G.R. wrote the paper.

## METHODS

### PROCESSING OF NEST CONTENTS

Macrophotographs of cells were taken with a Microoptics-USA photographic system equipped with an Infinity Photo-Optic K-2 lens system. Preserved larvae were first examined, and then head and body were separated and cleared by boiling in an aqueous solution of sodium hydroxide until cleared of opaque tissue. After being washed in water, the two parts were transferred to 70%–75% ethanol, stained with Chlorazol Black E, washed in ethanol, and submerged in glycerin on a well slide for study and storage. Microphotographs of them were taken with a Canon PowerShot A2300, 16.0 megapixels, hand held, through one lens of a Carl Zeiss compound microscope. Cocoons and cell walls, not requiring critical-point drying, were simply coated with gold/palladium and examined with a Hitachi S-4700 scanning electron microscope (SEM).

## CHEMICAL ANALYSIS

Entire brood cells collected in Israel were placed in 1.5 ml microtubes and shipped to the University of Neuchâtel for further analyses. Brood cells were opened and fragments of the cell wall were kept in  $-80^{\circ}\text{C}$  until analysis; some cell wall fragments were also kept at room temperature. In addition, the following tissues from the host plant *Heliotropium suaveolens* were collected from the field directly into methanol and shipped to Neuchâtel: leaves, trichomes from the leaves, and trichomes from the stems.

We performed chemical analyses of the cell walls, of the pollen provisions and of the source plant tissues using liquid injection and microvial headspace trapping techniques. For the liquid injections, samples were ground in liquid nitrogen and dissolved in 100  $\mu\text{l}$  dichloromethane. After agitation, centrifugation and addition of 10  $\mu\text{l}$  of a solvent solution containing two internal standards (200 ng of pure octane and 200 ng of nonyl acetate; Sigma-Aldrich Co. LLC.), a 2  $\mu\text{l}$  aliquot of the blend was injected into the gas chromatograph (GC) (injector parameters:  $280^{\circ}\text{C}$ , 9 psi pressure, 5 ml/min septum purge flow, total flow of 236.1 ml/min, purge flow to split vent of 230 ml/min at 1 min). A three-minute delay was set to allow the dichloromethane solvent to evaporate. Compounds were separated on an Agilent HP-1 MS column (30 m length  $\times$  250  $\mu\text{m}$  inside diameter and 0.25  $\mu\text{m}$  film thickness; starting temperature  $50^{\circ}\text{C}$  for 1 min, ramp of  $8^{\circ}\text{C}/\text{min}$  to  $250^{\circ}\text{C}$ , hold time 5 min, 2 min post-run at  $280^{\circ}\text{C}$ , helium at constant flow of 1.12 ml/min as carrier gas). Analyses were done with a GC Agilent 7890A coupled to a mass spectrometer (MS) Agilent 5975C with the following parameters: transfer line at  $280^{\circ}\text{C}$ , ion source and quadrupole set at  $230^{\circ}\text{C}$  and  $150^{\circ}\text{C}$  respectively; electron impact (EI) mode, ionization potential of 70 eV with scanning over the mass range of 33–500 amu.

The nest samples (cell walls and pollen provisions) could not easily be dissolved in methanol. For these substrates, components were extracted directly using microvial headspace technique. These were analyzed with identical GC-MS equipment as described above, and the system was equipped with both a thermal desorption unit and a cooled injection system (TDU and CIS; Gerstel GmbH). A sample of about 1 mg of the cell wall or of the pollen provision was placed directly in microvial inserts (Gerstel GmbH) then introduced into the TDU. In each case, the TDU was used in splitless mode, initially kept at  $50^{\circ}\text{C}$  for 30 sec then heated at  $640^{\circ}\text{C}/\text{min}$  to reach  $250^{\circ}\text{C}$  (hold time 5 min). During desorption, chemicals were trapped and cryo-focused ( $-80^{\circ}\text{C}$ ) in the CIS before being unfrozen at  $12^{\circ}\text{C}/\text{sec}$  to  $280^{\circ}\text{C}$  (hold time 8 min) and injected (solvent vent mode, vent pressure of 14 psi, vent flow of 50 mL/min, purge flow of 50 mL/min) into the column. The helium pressure was 14 psi (flow rate 1.65 mL/min) in constant flow mode. The temperature program of the GC operation was  $50^{\circ}\text{C}$  for 0.01 min, then increasing to  $250^{\circ}\text{C}$  at a rate of  $7^{\circ}\text{C}/\text{min}$  (hold time 5 min), followed by a 2 min post-run at  $270^{\circ}\text{C}$ .

Corresponding control analyses (identical solvent stored in identical vials and processed as our samples) for all samples were used to discern which compounds were not from studied material. In addition, a blank (pure solvent) was run between each type of sample in order to clean the system. For both types of analyses, particular attention was given to the presence of alkaloids and to obtain their preliminary identifications based on NIST11 mass spectral library (U.S. Department of Commerce), as well as PBM search format (Agilent Technologies, Inc.).

## DESCRIPTION OF NESTING SITE

During the summer and autumn seasons of 2011–2013, a population of *Haetosmia vechti* was observed on a vacant site surrounded by citrus groves and a busy road, on the outskirts of Rehovot, a city (population of 120,000) on Israel's central coastal plain (N 31°54'23" E 34°49'32", 58 m elev.) (fig. 1). The ground on this site consists of hamra (a type of sandstone prevalent throughout the coastal plain of Israel) covered by sand. The soil and vegetation on the site showed clear signs of anthropogenic disturbance, with artificial sand hillocks, construction waste, and ruderal and non-native plant species. Vegetation on the site was diverse; prominent taxa included *Heliotropium suaveolens*, *Centaurea procurrans*, and the invasive *Verbesina encelioides* (Cav.) A. Gray.

The first nest was found in October 2011, and three more in July and August 2013. All nests were located on the south-facing slope of a steep, artificial hillock of compacted sand about 1 m high (fig. 2). The surface was thinly vegetated, mostly *Verbesina* and *Centaurea* as well as dry, dead annuals. The soil was full of burrows and cavities used by various Hymenoptera (*Cataglyphis*, *Bembix*, *Nomioides*, etc.). Dozens of the forage plants (*H. suaveolens*) used by *Haetosmia* were located within a few meters to the south of the hillock. Three of the nest entrances were hidden under a live or dry plant (fig. 3), and one entrance was exposed (fig. 4). Nest entrances were accompanied by shallow, inconspicuous tumuli; they remained unplugged after nest completion.

The first two nests were discovered by locating a female, one excavating a burrow (fig. 5) and the other provisioning. We dug up the nests nine and 14 days later, when they were no longer active, and they yielded two and six cells, respectively. A third nest was found when no longer active, detected due to the repeated entry of a cuckoo wasp (Chrysidinae sp.); it produced three cells. The fourth nest was discovered while the female was excavating, and was dug up after eight days, when still active; it contained three completed cells and one incomplete cell. In all, 15 brood cells were obtained. We do not know whether a female builds more than one nest during its lifetime.

## NEST ARCHITECTURE

In two nests, females were observed kicking back with their legs to remove sand and pebbles from initiated burrows (fig. 5), suggesting that *H. vechti* females excavate their own nests rather than exploit preexisting burrows. Each nest consisted of a single open burrow, 3–4 mm in diameter, that descended diagonally downward from the entrance to a single enlarged chamber containing the brood cells, which was 5 to 15 cm below ground surface (fig. 6). Burrow and chamber walls were not lined or consolidated, and sometimes easily disintegrated upon excavation. The chamber contained an empty space above the cells, which probably serves as a work space for the female as she constructs and installs the cells. Cells were positioned side by side and adhered to one another, with the most recently constructed cell slightly elevated compared to the previous cell (figs. 7–9). Cells were grouped either in clusters (2 nests of 6 and 4 cells each; fig. 8) or in linear series (1 nest of 3 cells; fig. 7). Cells from the unfinished nest were much easier to separate, and one of their closures also separated quite easily. Cells were oriented with their long axis near to vertical, anterior (closure end) up. Hence, the rear ends of the cells were embedded in soil whereas their tops were exposed.



FIGURES 1–11. Nesting site and nests of *Haetosmia vechti* in Rehovot, Israel. 1. The sand hillock where nests were discovered is behind the person. 2. Part of the artificial sand hillock in which nests of *Haetosmia vechti* were discovered. The location of two adjacent nests is indicated by an arrow. 3. Female entering her burrow, concealed under dry leaves (arrow). 4. Exposed burrow entrance of *Haetosmia vechti*. 5. Female *Haetosmia vechti* digging a burrow. 6. An excavated nest with cell chamber (arrow). 7, 8. Two cell clusters from August 2013. 9. Cell cluster from October 2011 (with running ant). 10. Detail of cell wall from October 2011, showing needlelike structures characteristic of *Heliotropium*. 11. Dissected cell from October 2011, showing pollen mass and dead egg/larva on top.

## CELL STRUCTURE AND COMPOSITION

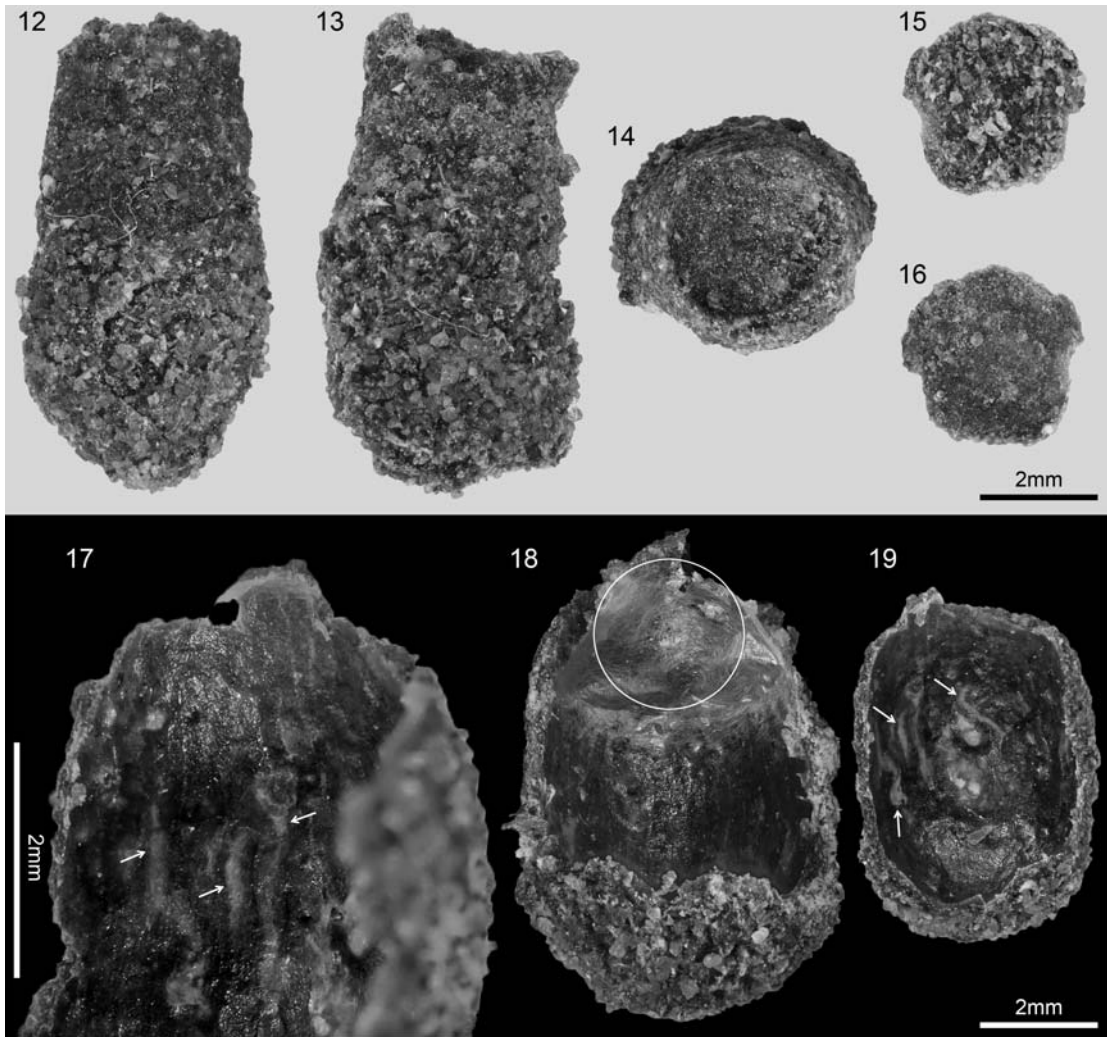
The cell of *H. vechti* appears to be bilaterally symmetrical, usually oriented with long axis vertical, so that externally in one view, the cell is urn shaped (fig. 12) and symmetrical, with a length of 7.0–8.5 mm (median 7.7 mm), a diameter of 3.2–3.85 mm (median 3.45 mm) at the anterior end, and with straight sides diverging outward to a maximum diameter of 4.1–4.6 mm (median 4.39 mm) at a point about 2 mm from the posterior end ( $N = 9$ , all measurements). Beyond that point the outline of the sides normally curves mesad to form a round posterior end to the cell. When that cell is rotated 90° on its long axis, the long axis is seen to curve, so that one side is slightly concave and the opposite side is strongly convex (fig. 13). Although the above description is presumably the fundamental external configuration of a cell, its shape is also modified by substrate inclusions (roots, pebbles), which probably account for the various asymmetries and peculiar angles in cell pictures (e.g., posterior end in fig. 13).

Cells from the 2013 nests had a dark green color externally (figs. 7, 8), whereas the cells from the 2011 nest had an overall sandy color (fig. 9), and their cell linings were found to contain whitish needlelike structures, closely resembling the trichomes covering the surface of Boraginaceae such as *Heliotropium* spp. (fig. 10). The cells' exterior surface appeared uneven with scattered imbedded grains of light and dark tannish quartz sand, set against a hard, darker matrix. The anterior end of the closed cell was encircled with a sharp rim (fig. 14) surrounding the externally concave closure, ca 1.0 mm deep at center below the rim crest. Several cells collected before cocoons had been spun revealed a finely pebbled, reflective inner cell wall at the front of the cell in the short area separating the feces-covered cell wall and the inner surface of cell closure. The inner surface of a cell closure was flat, extremely rough, and nonreflective (fig. 15), contrasting with a somewhat smoother exterior surface (fig. 16). It is obviously built into the cell wall, after the female completes storing the provisions and ovipositing. Cell provisions consisted of firm pollen masses at the base of each cell, taking up approximately two thirds of the cell's volume (fig. 11). Although based on incomplete observations of only two cells, it seems likely that provisions take on the shape of the lower part of a cell rather than being molded into a special form by the female.

The cell closure was examined in an attempt to determine the passageway or -ways for air exchange between the cell's interior and the outside world. Although no obvious openings were detected in the outer cell closure, it is assumed not to be airtight, which is probably true for all bee brood chambers, whether or not the species are cocoon spinners. The fact that cocoons in so many families have small screened openings normally restricted to the front of their cocoons thereby allowing air exchange is further evidence of the air-exchange function through cell closures (e.g., Colletidae: Diphaglossinae, Rozen, 1984: figs. 24–33; Melittidae: *Macropis*, Rozen and Jacobson, 1980: figs. 6, 10; Megachilidae: Lithurginae, Rozen, 2013: figs. 13–17,<sup>8</sup> and Megachilinae, Rozen and Hall, 2011: figs. 17, 43; Apidae: Apinae, Rozen and Buchmann, 1990: figs. 18, 27–34; Straka and Rozen, 2012: figs. 16–19; Michelette et al., 2000: figs. 7, 10, 11, 13).

Initial examination of the nests suggested that the embedding matrix was resin, because the cells found in 2011 were yellow-brown and not green as in the case of osmiine nests made of pure masticated leaf material. However, when heated on a heating plate, this "glue" did not

<sup>8</sup> In *Lithurgus*, filter apertures occur toward the rear of cocoons.



FIGURES 12–19. Macrophotographs of cells of *Haetosmia vechti*, anterior ends at top when in lateral view. 12. Entire cell, exterior surface, showing urn shape. 13. Same cell as figure 12 turned 90°, showing asymmetry. 14. Cell top, showing elevated rim surrounding closure. 15. Cell closure, inner surface. 16. Same closure as figure 15, exterior surface. 17. Inner surface of cell showing transparent cocoon covering dark feces and streaks of white material (arrows); scale bar at left. Note ribbons of feces best seen at anterior end. 18. Another opened cell with anterior end of cocoon still attached with filter (circle) identified. 19. Another cell showing inner surface, with white material (arrows); scale bar for figures 18, 19 at lower right.

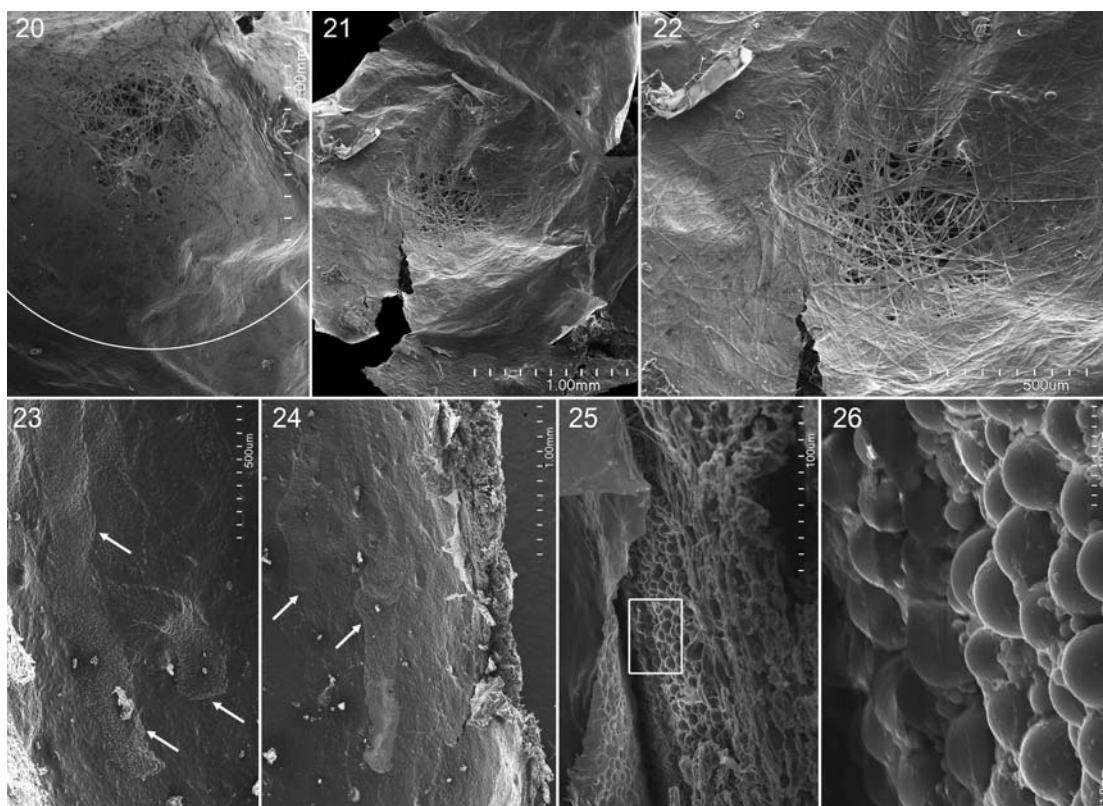
melt, nor did it burn when passed through a flame. For comparison, fragments of a nest of the resin bee *Rhodanthidium siculum* Spinola quickly melted when put on a heating plate and burned when passed through a flame. The fragments from the *Rhodanthidium* nest did not dissolve in water, even when heated to boiling point. In contrast, nest fragments of *Haetosmia* dissolved in water at room temperature after 5–10 minutes. What appeared as a brittle, hard matrix turned into a soft, fiberlike mass that could easily be torn by bending after soaking in water. In the yellow nests of 2011, this softened mass was colorless to whitish, whereas in the green nests of 2013, this softened mass was green. When taken out of the water, the mass dried

and became brittle again. From these observations, we conclude that both the sandy colored nests collected in 2011 and the green nests from 2013 are built with masticated leaf pulp to which the bee adds pebbles and sand grains. Indeed, during August 2013, females were observed chewing off and masticating the green leaves of *Centaurea procurrens* (fig. 28).

GC-MS analysis revealed the presence of specific pyrrolizidine alkaloids that are typical secondary compounds of the plant family Boraginaceae, in all analyzed cell wall fragments (fig. 27). The precise identification of each alkaloid was not possible, but the following compounds were identified with high probability based on the NIST11 mass spectral library: heliotrine, rivularine, and heliosupine (echinatine and echimidine were also found, though at much lower concentrations; fig. 27). All these alkaloids are exclusively found in the family Boraginaceae, and at least heliotrine has been previously identified in *Heliotropium suaveolens* (Güner, 1986). The three main alkaloids were found in cell wall fragments from the nests found in both 2011 and 2013. We also identified the same alkaloids in the leaves and the trichomes (both on leaves and stems) of *H. suaveolens* collected from the nesting site in Israel. Lastly, the pollen provisions also contained high amounts of these alkaloids. These results indicate with confidence that plant material used for cell construction comes (at least in part) from the Boraginaceae family. Given that *Heliotropium suaveolens* was the main species of Boraginaceae present in the nesting site (some *Echium angustifolium* Mill. was also present) and that the bees are fully dependent upon the presence of this genus for pollen foraging (see below), these analyses suggest that the bee had most likely collected nesting material from this plant. The presence of the same alkaloids in the green cells collected in 2013 suggests that females may have mixed leaf pulp collected from *Centaurea* with some plant material collected from *Heliotropium* too. In 2011, the observed nest was active late in the season, when *Centaurea* plants were dry and could not be used by the bee. Hence, the source of leaf pulp was probably mostly from *Heliotropium* in 2011, as demonstrated by the presence of numerous trichomes in the nesting material, and *Centaurea* in 2013, possibly explaining the difference in color. It seems unlikely that the high alkaloid concentrations detected in the nest fragments were due to contamination from the pollen provisions. The presence of high concentrations of toxic alkaloids in the pollen provisions is surprising, but not unusual, given that alkaloids have been detected in the pollen of other Boraginaceae (Boppré et al., 2005).

### COCOON STRUCTURE

The sides and posterior part of the cocoon consist of a single, very thin, transparent sheet of silk (fig. 17) applied over parallel ribbons of mostly dark tan feces that have been pressed against the cell wall paralleling the long axis of the cell. The silk is usually colorless, but one cocoon was tinted a reddish tan. Because of the transparency of the silk viewed from inside (note lack of fibrous texture on silk except at the anterior end close to the filter area, fig. 18), the thin fecal layer is visible with scattered elongate smudges of opaque white material showing through (figs. 17, 19, 23, 24, arrows). In a case where silk was accidentally removed, the stark white streak contrasted sharply with the very dark feces. The feces are applied to the entire cell wall except for the cell closure and often for a short section of cell wall surrounding the closure. The source and nature of the white material is unknown but likely relates to a late fine-grained white or yellowish anal discharge reported in the



FIGURES 20–26. SEM micrographs of cells and cocoons of *Haetosmia vechti*. **20.** Front end of cocoon in figure 18, showing filter area. **21.** Filter area of another cocoon. **22.** Same, close-up, showing surrounding fibrous area. **23, 24.** Inner cocoon surface demonstrating: (1) transparency of silk surface; note debris seeming to “float” on surface and (2) the near disappearance of elongate streak of white material (arrows), which reflects shape and not color; figure 24 with cocoon on left and edge of wall with thin fecal layer and resin wall on right. **25.** Another area of cell wall with cocoon on left, showing cluster of presumed microorganisms on inner surface of wall beneath cocoon and impression of organism indicated on undersurface of cocoon. **26.** Close-up of microorganisms, suggesting that more than one species may be involved.

case of other megachilid larvae (e.g., Rozen and Hall, 2012). Because feces are not incorporated into the cocoon, silk production does not start until after defecation, nor is there any silk production before defecation, as in some Rophitini (Halictidae) (Torchio et al, 1967) and at least some Tapinotaspidini (Rozen and Michener, 1988). Occasionally one can see clusters of microorganisms on the surface of the feces just under the cocoon fabric (figs. 25, 26).

Under high magnification, the inner surface of the transparent silk of most of the cocoon irregularly glistens because of variable attachment to fine grain feces and the scattered appearance of short strands of fibrous silk. At its anterior end where there are no feces, the cocoon is separated from the cell surface so that a small mostly open space occurs between the front end of the cocoon and the cell closure. When seen from inside, the cocoon fabric, no longer applied to the cell wall, is changing from a nearly clear cellophanelike transparency to a whitish, more or less opaque surface because the silk strands are no longer fused but become fibrous (fig. 18). The mostly open space is actually occupied by an open network of silk strands supporting the

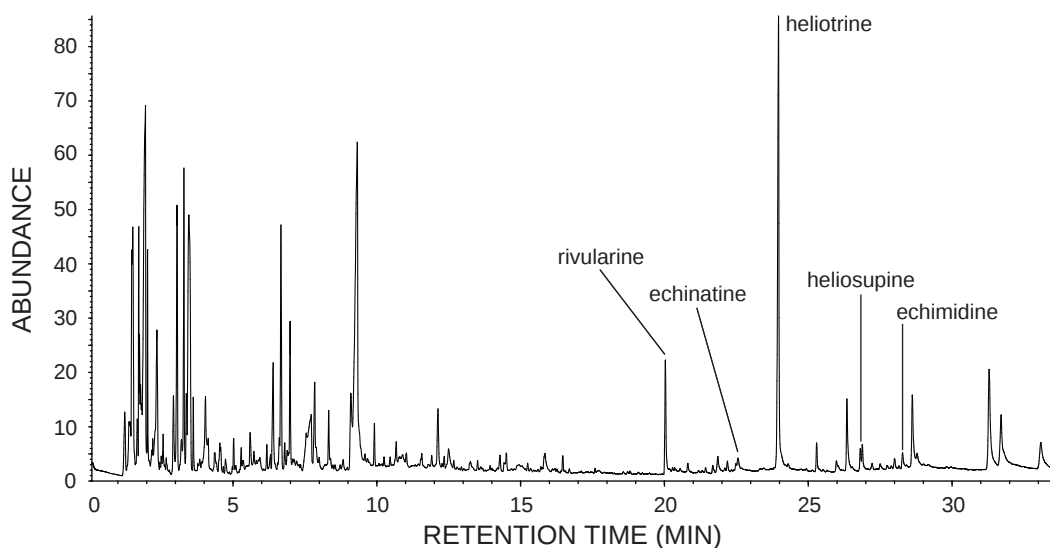


FIGURE 27. Representative chromatogram of the chemicals included in fragments of the cell wall of *Haetosmia vechti*. The compounds were analyzed using microvial and headspace technique, as described in the text. Peaks corresponding to the main pyrrolizidine alkaloids found in the substrate are labeled. These alkaloids are typical of the plant family Boraginaceae.

fabric at the front of the cocoon. Near the center, the anterior end of the cocoon becomes so fibrous that irregular-sized screened openings occur, presumably allowing air exchange between inside the cocoon and ambient air (figs. 18, 20–22) while still excluding possible parasites.

#### FLORAL PREFERENCES OF *HAETOSMIA* SPP.

To uncover the degree of pollen host specialization in *Haetosmia* spp., we microscopically analyzed the scopal pollen contents of 68 female museum specimens of *Haetosmia brachyura* (Morawitz) (3 samples from 3 localities in “Turkestan”), *H. circumventa* Peters (54 samples from 11 localities in Morocco, Israel, and the United Arab Emirates), and *H. vechti* (11 samples from 3 localities in Israel) by applying the methodology of Sedivy et al. (2013b). All 68 pollen loads consisted exclusively of pollen of *Heliotropium* (Boraginaceae), which suggests that the three *Haetosmia* species are most probably all narrowly oligolectic on this plant genus. This finding is in line with Mavromoustakis (1954), who considered *H. vechti* to be specialized on *Heliotropium*, and with field observations of *H. vechti* in Iran (C.P., C.S.) and Israel (A.G., C.P., G.P.), *H. brachyura* in Uzbekistan (C.P.), and of *H. circumventa* in southern Morocco (A.M., C.S.) and Israel (C.P., C.S.), where both males and females were observed to forage only on flowers of different *Heliotropium* species (figs. 29–30) despite a diversity of flowering species occurring nearby. Flower records of *H. brachyura* on *Salsola richteri* (Moq.) Karel ex Litv. (Amaranthaceae) and of *H. circumventa* on *Neurada procumbens* L. (Neuradaceae) and on *Gymnocarpus decander* Forssk. (Caryophyllaceae) (Popov, 1960; Peters, 1974; A.G., unpubl. data) suggest that flower taxa other than *Heliotropium* are occasionally also exploited, but probably for nectar only.

In the females of all three *Haetosmia* species, the second segment of the labial palpus is densely covered with rather long, apically curved, and capitate bristles (figs. 31–32). These special-

ized bristles of peculiar spoonlike shape were hypothesized by Peters (1974) to be an adaptation to collect *Heliotropium* pollen, which is hidden inside the narrow corolla tube. Recent field observations in southern Morocco in 2008 (A.M.) and in Israel in 2010 (C.S.) support this hypothesis: flower-visiting females of *H. circumventa* inserted their proboscises into the *Heliotropium* flowers before they repeatedly moved them slightly up and down inside the corolla tube (fig. 30). This behavior clearly indicates that the specialized bristles indeed serve to extract pollen from within the *Heliotropium* flowers. How females remove the pollen from the proboscis could not be observed, but likely happens in flight immediately after leaving the flower with the aid of the broadened basitarsi of their forelegs, as previously suggested by Peters (1974).

Interestingly, morphologically very similar bristles of spoonlike shape are developed on the labial palpi in females of other Palaearctic osmiine bee species that collect pollen of *Heliotropium*, such as the closely related *Hoplitis persica* (Warncke) and *H. premordica* Griswold (Warncke, 1991; Griswold and Michener, 1998; Sedivy et al., 2013b), *Protosmia hamulifera* Griswold (Griswold, 2013), and two undescribed species of *Protosmia* (A. Müller, unpublished). The amazing similarity in shape and location of these bristles among the three distantly related osmiine bee taxa suggests that they are highly adaptive for effectively extracting pollen out of the *Heliotropium* flower tubes. Flowers of *Heliotropium*—a plant genus of worldwide distribution—are also pollen hosts of non-Palaearctic bees, such as *Geodiscelis megacephala* (Colletidae, Xeromelissinae) and *Teratognatha modesta* Oglobin (Apidae, Exomalopsini) from Argentina and several unrelated *Calliopsis* and *Calonychium* species (Andrenidae, Calliopsini) from both North and South America (Michener and Rozen, 1999; Rozen, 2011). Similar to *Haetosmia*, in all these species the mouthparts or forelegs (in *G. megacephala*) are equipped with highly specialized bristles, which are evidently used to pull pollen out the *Heliotropium* flowers (Michener and Moure, 1957; Michener and Rozen, 1999; Rozen, 2011). Thus, pollen-harvesting bristles to specifically exploit flowers of *Heliotropium* have independently evolved several times on different continents in bees of four families.

## IMMATURE STAGES

The 14 completed cells from all nests were dissected 0 to 6 days after collection, and yielded as follows: 1 freely emerged female adult, 2 male and 4 female pupae, 2 postdefecating larvae, 1 defecating larva + smaller parasitic larva (Sapygidae?), 1 parasitic larva (Bombyliidae?), 1 provision with dead egg or young larva, 1 provision with no offspring, and 1 unidentified, dead larva + pupa. Even though one nest was harvested when still active and its contents dissected within hours, offspring from all nests were found at rather advanced stages of development, except for one dead egg/early instar that failed to develop in the 2011 nest (fig. 11). Hence, descriptions of eggs and early instars are lacking.

## DESCRIPTION OF MATURE LARVA

Figures 33, 35–38

**DIAGNOSIS:** The single feature that seems to distinguish the mature larva of *Haetosmia vechti* from all other known megachilid larvae is the apparent absence of an outer ring of the

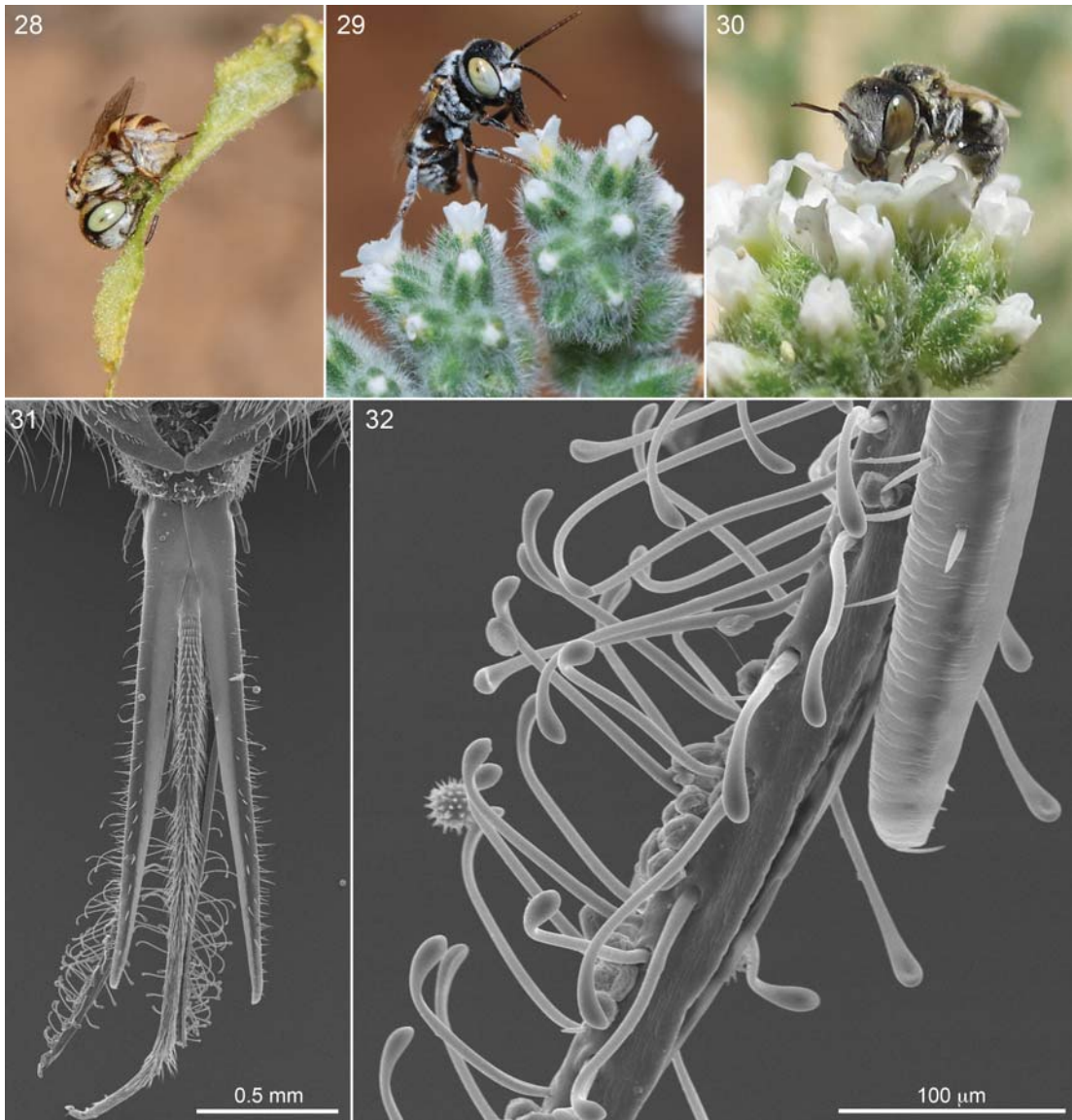
antenna on two of the only three larvae collected,<sup>9</sup> both of which had been cleared and stained for study. Almost certainly the ring is actually not absent but not sufficiently expressed because of the thinness of the surrounding parietal cuticle, and that might therefore either be a condition of the integument as it approaches molting or some other ephemeral developmental stage, or be associated with the very small body size of the mature larva.

In other respects, the mature larva of *Haetosmia vechti* exhibits the basic larval megachilid features including: abundant body setae; transverse labral sclerite; reasonably massive mandibles with broad, parallel sided apex bearing two apical teeth; strongly projecting labiomaxillary region with prementum and postmentum clearly divided and with well-developed projecting lips on transverse salivary gland opening; and anus positioned near top of abdominal segment 10. However, as pointed out by Michener (1953), mature larvae of Megachilidae tend to be homogenous, with slight differences between taxa and with few characters that indicate affinities within the family. The following attempts to separate the mature larva of *H. vechti* from those of some other osmiine genera.

Known larvae of *Osmia* tend to have a denser body vestiture (setae and spicules) than *Haetosmia vechti*, as measured by the number of setae/spicules on the lateral lobe of abdominal segment 8. Thus, *H. vechti* has fewer than 10 setae, and the following have many more setae (or setiform spicules) (actual number in brackets when known): *Osmia* (*Ozbekosmia*) *avosetta* Warncke (Rozen et al., 2010) [80]; *O. (Helicosmia) chalybea* Smith (Rozen and Hall, 2011) [ca. 40]; *O. (O.) l. lignaria* Say (Baker et al., 1985) [>15]; [*O. (Melanosmia) nigrobarbata* Cockerell [28, new data]; *O. (O.) ribifloris* Cockerell [ca. 75, new data] (specimens in AMNH collection). However, *Hoplitis* (*H.*) *monstrabilis* Tkalců with only 5 setae on the lateral lobe of its abdominal segment 8 (Rozen et al., 2009) agrees with *H. vechti*, although its larger body size and more slender mandibular teeth (ibid.: figs. 11, 17) will easily separate its mature larva from that of *H. vechti*. According to Baker et al. (1985: fig. 3A), *Hoplitis* (*Monumetha*) *spoliata* (Provancher) (as *H. (Andronicus) cylindrica* (Cresson)) has fewer than 10 setae on the lateral lobe of abdominal segment 8. *Hoplitis* (*Hoplitis*) *adunca* (Panzer) with 13 setae pictured on the lateral lobe of abdominal segment 8 (Grandi, 1961: fig. 371 #2) is slightly hairier than other congeneric species, but in other respects seems indistinguishable from them. However, the larger body size of these two species will again distinguish them from *H. vechti*. Since adults of other species of *Hoplitis* are substantially smaller, size alone cannot be considered a diagnostic character separating *Haetosmia* from *Hoplitis*. In the case of *Heriades* (*Heriades*) *crenulatus* Nylander, (as *Eriates crenulatus* Nyl., Grandi, 1961: fig. 365 #2), with 6 setae on the lateral lobe of abdominal segment 8, body size may be a more difficult criterion since adult body size approaches that of *Haetosmia vechti*. In the final analysis, osmiine larvae, to the extent known, are a homogenous group.

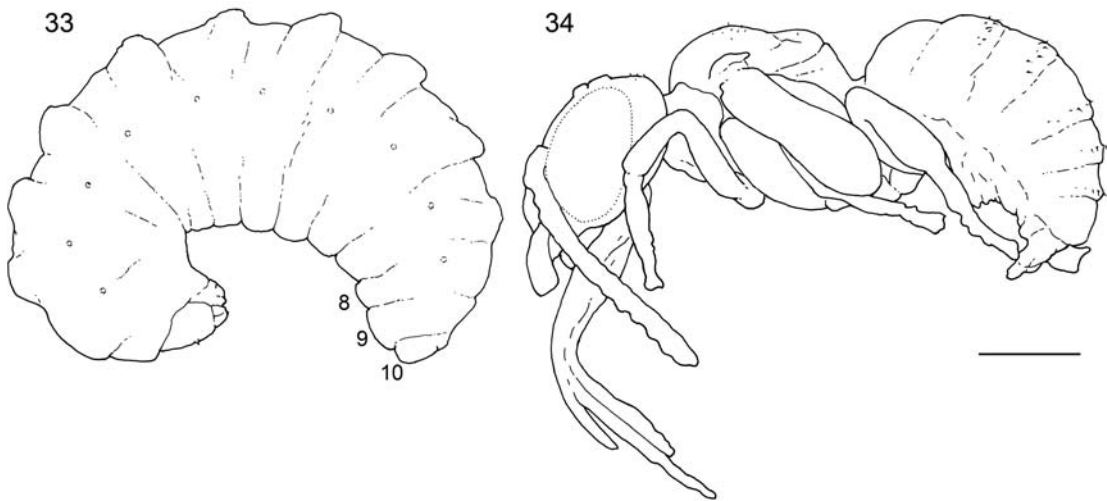
**DESCRIPTION: Head:** Setae moderately long but rather scarce on head capsule; those of maxillary and labial apices abundant, tending to be slightly curved, forward projecting, and conspicuous, with large, pronounced alveoli. Head capsule unpigmented except at points of articulation on internal head ridges by mandibles and cardines. Spiculation restricted to lateral lobes of hypopharynx, not on maxilla. Area immediately above hypostomal ridge and just behind posterior

<sup>9</sup> The third larva was harvested from a cell frozen intact at -80° C for chemical analysis, and therefore its morphology could not be studied.



FIGURES 28–32. Adult *Haetosmia*. **28.** Female of *Haetosmia vechti* masticating a leaf of *Centaurea procurrens*. **29.** Male of *Haetosmia vechti* feeding on flowers of *Heliotropium*. **30.** Pollen-collecting female of *Haetosmia circumventa* on *Heliotropium* sp. **31.** SEM micrograph of female proboscis of *Haetosmia vechti* with specialized pollen-harvesting bristles densely covering the second segment of both labial palpi. **32.** SEM micrograph of pollen-harvesting bristles on the right labial palpus of *Haetosmia vechti*.

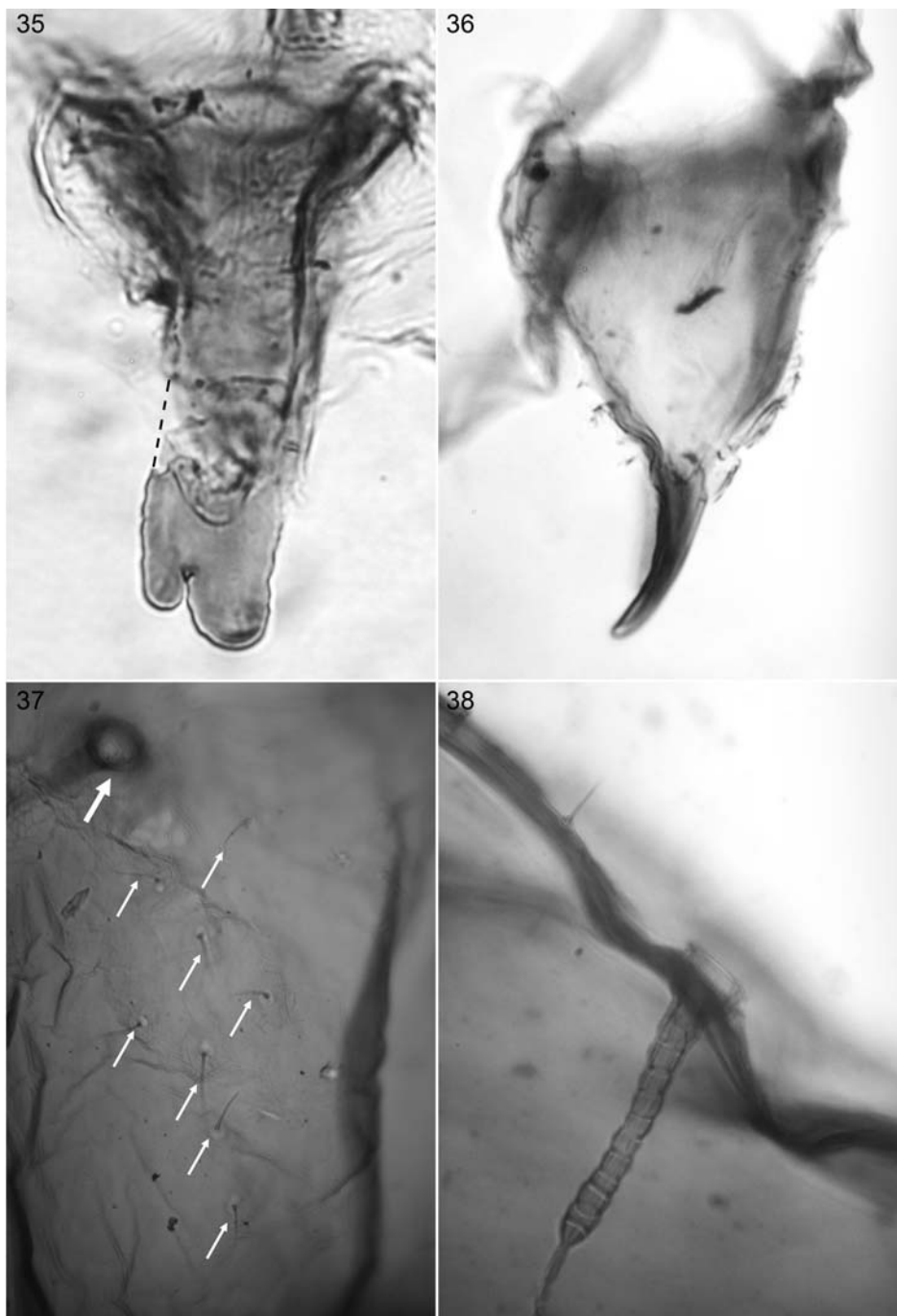
mandibular articulation not produced as downward-directed tubercle as present in many *Coelioxys* (Rozen and Kamel, 2007: fig. 47). Coronal ridge virtually absent; postoccipital ridge well developed, clearly bending forward toward median line on top of head; hypostomal ridge well developed, giving rise to pronounced dorsal ramus that extends posteriorly from middle of ridge nearly to postoccipital ridge; both hypostomal ridge and ramus darkly pigmented; posterior part of ridge bending strongly mesad, forming deeply recessed posterior tentorial pit at junction with



FIGURES 33-34. Diagrams of immatures of *Haetosmia vechti*, lateral views, drawn to same scale = 1.0 mm. **33.** Postdefecating larva, demonstrating overall shape; vestiture not indicated. The specimen used for this illustration did not preserve well, so that the illustration lacks accuracy, although overall form is correct; the description is believed to be far more reliable. **34.** Male pupa with vestiture indicated.

posterior tentorial bridge; pleurostomal ridge well developed but not strongly pigmented; epistomal ridge well developed from anterior mandibular articulation to anterior tentorial pit; from pit, ridge extending vertically until fading out above level of antennal papilla, hence not extending across to opposite side of head. Tentorium moderately robust including dorsal arms. Parietal bands scarcely evident. Diameter of basal ring of antenna unknown because ring not evident; antennal papilla faintly pigmented, slender, gradually, evenly tapering apically, about twice as long as basal diameter, bearing perhaps three tightly clustered sensilla on narrow apex. Lower margin of clypeus angled upward at midline, so that at midpoint margin nearly at level of anterior tentorial pits. Labral sclerite transverse, pigmented.

Mandible (figs. 35, 36) moderately robust; apex darkly pigmented, bidentate with ventral tooth longer than dorsal tooth; both teeth on defecating larva broadly rounded, on postdefecating larva teeth more narrowly rounded; dorsal apical edge of dorsal tooth faintly, irregularly uneven subapically; ventral apical edge of ventral tooth also uneven; apical concavity defined basally, but weakly so; cuspal area not developed; outer surface with single conspicuous long seta or tubercle. Maxillary apex strongly bent mesad in frontal view, so that maxillary palpus subapical in position; cardo distinct, pigmented, posterior end directed toward posterior tentorial pit; stipes a conspicuous long, sclerotized, pigmented rod, at posterior end articulating with cardo, at anterior end broadening and giving rise to distinctly pigmented articulating arm of stipes, best seen on cleared specimen; maxillary palpus faintly pigmented, moderately small, about same size as antennal papilla and labial palpus, which is also faintly pigmented. Labium clearly divided into prementum and postmentum; apex moderately narrow; premental sclerite distinct, weakly sclerotized; postmentum nonsclerotized. Salivary lips projecting, transverse, width distinctly less than distance between bases of labial palpi. Hypopharynx consisting of two widely separated lateral lobes, which are spiculate.



FIGURES 35–38. Microphotographs of last larval instar of *Haetosmia vechti*. 35, 36. Right mandible, inner and ventral views, respectively. 37. Lateral lobe of abdominal segment 8 left side, showing spiracle and 8 setae (arrows). 38. Spiracle side view, showing elongate subatrium with barrel-shaped chambers.

**Body** (figs. 33, 37, 38): Body vestiture apparently consisting of only one form: long seta, tapering to fine, often curved point, arising from distinct alveolus; these setae conspicuous dorsally and laterally on thorax and abdomen; area of lateral lobe of abdominal segment 8 (i.e., area below level of spiracle) with approximately 8 setae (fig. 37); integument throughout without spicules. Body form moderately robust; in lateral outline (fig. 33) abdominal segments 3–5 with greatest diameters; segments 6–10 tapering posteriorly; intersegmental lines moderately weakly incised on postdefecating larva; intrasegmental lines not evident; paired body tubercles absent; middorsal body tubercles evident on midbody segments; lateral lobes (sometimes called “ventro-lateral tubercles,”; Michener, 1953; Baker et al., 1985) scarcely evident; integument of lateral lobe of abdominal segment 8 (i.e., area below spiracle, fig. 37) bearing 10 or fewer setae and no spicules or setiform spicules; venter of abdominal segment 9, slightly produced, so that abdominal segment 10 attached somewhat above middle of segment 9 in lateral view; anus positioned toward top of segment 10; integument below anus with patch of short setae more or less direct toward anus. Spiracles unpigmented, subequal in diameter; atrium globular with width not much greater than depth, projecting above body wall, with rim; peritreme narrow, so that diameter of atrial opening as much as four times peritreme width; atrial inner surface with rows of wrinkles concentric with primary tracheal opening; atrial wall also with concentrically directed spicules; primary tracheal opening with collar; subatrium long with 10 to 20 chambers, but shape of chambers variable as discussed under Remarks, below. Sex characters unknown.

**MATERIAL EXAMINED:** One defecating larva (#5), one postdefecating larva (#12): Israel: Rehovot, August 20 and 22, 2013, respectively (A. Gotlieb, G. Pisanty).

**REMARKS:** When first describing the postdefecating larva, J.G.R. found that the subatrium was remarkably different from those of *Osmia chalybea* and initially described it as follows “subatrium long with 10 to 20 chambers, almost all of which are barrel shaped (no external constriction between chambers)” with the intent to describe figure 38. However, after doing so, he examined the cleared spiracle on the defecating larva of *Haetosmia*, which had a shorter subatrium with normally strong constriction between chambers. The pronounced difference between these two larvae appears to be an ontogenetic change, which has not been reported before for any bee larva. Because the subatrium appearance (though longer) corresponds to that of *O. chalybea* (Rozen and Hall, 2011; fig. 79), might the elongate configuration with barrel-shaped chambers be preparatory for pupal development? Such a phenomenon has not been noted before, but the presence of so many pupal *H. vechti* in nests at this site suggests the possibility.

Eickwort (1973) wisely observed with respect to larval *Hoplitis* (*Hoplitis*) *anthocopoides* (Schenck) “the mandibular teeth vary considerably from long and narrowly rounded in newly moulted” individuals “to short and much worn in mature larvae, so their length is not a valuable taxonomic characteristic.” To this might be added another variable: shape of the apical mandibular teeth.

#### PUPA

Figure 34

**DIAGNOSIS:** As indicated by McGinley (1989), pupae of *Osmiini* have been little studied. The pupa of *Haetosmia vechti* can be distinguished from those of *Osmia* (*Pyrosmia*) *submicans*

Morawitz described by Moustafa and Berry (1976: figs. 4, 6A)<sup>10</sup> and *Hoplitis* (*H.*) *anthocopoides* treated by Eickwort (1973: fig. 37) in that it lacks a band of setae on T1. The following also have at least some setae on T1 based on specimens in the AMNH: *Osmia* (*O.*) *cerinthidis* Morawitz, *Osmia* (*Helicosmia*) *chalybea* Smith, *Osmia* (*Melanosmia*) *nigrobarbata* Cockerell, and *Osmia* (*O.*) *ribifloris* Cockerell. The fact that the setae on T1 tend to be reduced in numbers and lengths compared with setae and the following terga suggests that presence or absence of setae may not prove to be an entirely reliable difference.

Another feature that may be distinctive is the small size of setae and their associate tubercles found on the vertex and mesoscutum, contrasting with the somewhat more pronounced tergal setae. This, however, may be more obvious for males rather than females.

The following description is formatted after pupal descriptions in Rozen and Kamel (2007).

**Entire Body Length:** 5.2–6.0 mm ( $N = 6$ ).

**Head:** Posterior part of vertex with scattered very small erect setae arising from small tubercles, these setae scarcely reaching posterior ocelli; front of head lacking tubercles and verrucae. Discal area of labrum moderately protuberant (accommodating erect adult setae) as seen in lateral view (fig. 34); pupal ocelli well defined. Mandible simple, without obvious swellings.

**Mesosoma:** Posterior half of mesoscutum with inconspicuous, scattered small setae, many rising from small tubercles about same length as their setae; mesoscutellum with one or two small setae on each side. Lateral angle and lateral lobe of pronotum strongly projecting. Mesoscutum and mesoscutellum without tubercles or verrucae; axillae weakly defined (fig. 34); metanotum without swelling; area around propodeal spiracle apparently slightly swollen; mesepisternum without tubercles. Tegula not produced, without tubercles or verrucae; wings without tubercles. All coxae with low apical tubercle; foretrochanter with small apical tubercle; other leg segments little modified.

**Metasoma:** Terga except for T1 with subapical transverse bands of conspicuous, posteriorly directed setae (fig. 34) that are somewhat longer and stouter than those of mesoscutum, some rising from small tubercles; these setae most pronounced sublaterally on male T2–T7 and female T2–T6; T6 not elongate in male, so that not extending beyond T7 in lateral view (fig. 34) as in *Coelioxys*; terminal spine questionably present as small, dorsally directed tubercle in one male, otherwise presumably not developed. Sterna without conspicuous setae.

**MATERIAL STUDIED:** Two male and four female pupae in various stages of pigmentation from same locality and dates identified for larvae.

**REMARKS:** Differences between pupae of males and females go beyond anatomical differences in metasomal feature associated with developing sex organs and scopae or lack thereof, as follows: Male antennae much longer, extending to posterior end of mesosoma; in female, antennae ending at mandibular apex. Female mandible widening into broad apex that will accommodate tridentate adult mandibular apex; male mandible extending without widening toward truncated apex that accommodates bidentate adult mandibular apex. Male forebasitarsus about as wide as foretibia; female forebasitarsus somewhat swollen compared with broadest part of foretibia.

<sup>10</sup>Note Moustafa and Berry (1976) used the notation T1, T2, etc., to refer to *abdominal* terga even though they used the term “metasoma” in their description, as evidenced by their figure 4B where they labeled the first metasomal tergum II T. (i.e., T2) and figure 6A where they labeled the propodeum I T. (i.e., T1).

## DISCUSSION

Our results, together with the phylogenetic position of *Haetosmia* and the detailed analyses of the nesting biology in the genus *Hoplitis* (Sedivy et al., 2013a), suggest that the biology of *Haetosmia* mirrors the ancestral nesting biology within the *Osmia* group: brood cells made of masticated plant material placed in burrows excavated by the females in the ground. As indicated by Sedivy et al. (2013a), most ground-nesting Osmiini are restricted to desert or Mediterranean climates. The fact that the cell walls of *H. vechti* quickly dissolved in water would probably prevent this species from maintaining viable populations under less arid temperate climates, where rain events during the nesting season might damage the cells. Two possible adaptations may have enabled diversification of the Osmiini in wetter regions. First, gradual changes in the nesting substrate may protect the nests from humidity. Numerous osmiine lineages nest in various cavities such as rock crevices or above-ground cavities such as pithy stems or existing burrows in wood. An astonishing diversity of Palearctic Osmiini in unrelated lineages also nest in empty snail shells (Müller, 2014). In fact the Mediterranean habitats bordering the desert regions of the Palearctic exhibit an unusual abundance of snails, and it is possible that nesting in empty snail shells represents an adaptation to protect the nests from moisture. Second, some Megachilidae incorporate labial gland secretions into their nests. Kronenberg and Hefetz (1984) demonstrated that the very resistant nest of the dauber bee *Megachile (Chalicodoma) sicula* (Rossi) was made hydrophobic through the incorporation of labial gland secretions. The presence of secretions is unlikely in *Haetosmia* given the solubility of the brood cell matrix in water. However, our chemical analyses of the cell wall would probably fail to detect such secretions, as Kronenberg and Hefetz (1984) indicate that the secretions could be detected only when the fresh, moist nest material was placed into methanol. Whether osmiine bees include secretions in their nests, remains poorly known. *Hoplitis (Hoplitis) anthocopoides* and its relatives build very resistant, hydrophobic, exposed nests highly similar to those found in the dauber bees of the *Megachile* subgenus *Chalicodoma* (Eickwort, 1973; Westrich, 1990), although the chemical nature of these nests has never been investigated. Secretions have been suggested to be present in the mud cell walls of *Osmia (Osmia) bicornis* (Héroin-Delaunay, 1966). Further research on this diverse group of bees is needed, and will shed more light on the complex evolution of bee nesting behavior.

## ACKNOWLEDGMENTS

McGinley's (1989) catalog of immature Apoidea was a useful guide to literature dealing with descriptions of immature Osmiini.

Stephen Thurston, Senior Scientific Assistant, AMNH, took the macrophotographs of cells and arranged all plates of illustrative material for this publication. Eli S. Wyman, Curatorial Assistant, AMNH, prepared immature specimens for SEM examination, produced all SEM images, and edited the text. Comments by Laurence Packer and an anonymous reviewer improved the manuscript.

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