

## Research

# Linking the Morphology of Sternal Glands to Rubbing Behavior by *Vespa soror* (Hymenoptera: Vespidae) Workers During Recruitment for Group Predation

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## Abstract

The activities of social insect colonies are supported by exocrine glands and the tremendous functional diversity of the compounds that they secrete. Many social wasps in the subfamilies Vespinae and Polistinae have two sternal glands—the van der Vecht and Richards’ glands—that vary in their features and function across the species in which they are found. Field observations suggest that giant hornets use secretions from the van der Vecht gland to chemically mark targeted nests when workers initiate group attacks on social insect prey. However, descriptions of giant hornets’ sternal glands and details about their recruitment behavior are lacking. We describe the morphology of the sternal glands of the giant hornet *Vespa soror* du Buysson and consider their potential to contribute to a marking pheromone. We also assess the gastral rubbing behavior of workers as they attacked *Apis cerana* F. (Hymenoptera: Apidae) colonies. *V. soror* workers have well-developed van der Vecht and Richards’ glands on their terminal gastral sternites, with morphologies that robustly support the synthesis, storage, and dissemination of their secretory products. Observations confirm that the van der Vecht gland is exposed during gastral rubbing, but that the Richards’ gland and glands associated with the sting apparatus may also contribute to a marking pheromone. Workers briefly but repeatedly rubbed their gasters around hive entrances and on overhead vegetation. Colonies were heavily marked over consecutive attacks. Our findings provide insight into the use of exocrine secretions by giant hornets as they recruit nestmates to prey colonies for group attacks.

**Key words:** exocrine gland, pheromone, recruitment signal, predator–prey interaction, scent marking

Glandular secretions play a prominent role in the lives of social insects, as evidenced by the discovery over the last century of an impressive number of exocrine glands in bees, ants, wasps, and termites (Cassier and Lensky 1997, Billen and Morgan 1998, Billen 2009, Gonçalves et al. 2010, Billen and Šobotník 2015, da Silva et al. 2021). Exocrine glands store their products and release them outside of an animal’s body (Noirot and Quennedey 1974). However, this simple description belies the astonishing variety of specialized

exocrine glands found among social insects, reflecting the breadth of their behavioral diversity and the complexity of social life. Many exocrine glands produce conspicuous materials with structural or physical functions, such as the defensive secretions discharged at enemies by nasute termite soldiers (Noirot 1969, Quennedey 1984, Santos and Costa-Leonardo 2006) or the construction materials produced by the wax gland complexes of eusocial corbiculate bees (Cruz-Landim 1963, Noll et al. 2021). However, a major function

of exocrine glands is to produce chemical signals that organize collective phenomena (Billen and Morgan 1998, Billen 2011).

Pheromones and the exocrine glands that produce them enable chemical communication among social insects, providing a window into the proximate mechanisms that underpin their incredible evolutionary success (Hölldobler and Wilson 1990, Downing 1991, Billen and Morgan 1998, Wilson and Hölldobler 2005). Because of their central role in the lives of eusocial insects, the presence and ultrastructure of exocrine glands, as well as the social deployment of their products, can provide useful clues about phylogenetic relationships among related groups (Billen 1994, Mitra 2013, Noll et al. 2021). Innovative uses of exocrine glands can also support the diversification of unique lifestyles among eusocial insects. This is especially true for exocrine glands that are found only in a specific taxonomic group. The study of exocrine gland structure and function in the eusocial wasps of the family Vespidae fits this interesting scenario.

The vespids are a widespread group of over 5,000 aculeate wasp species that span the spectrum of sociality from solitary to eusocial (Piekarski et al. 2018). Because they provide extant examples of highly diverged forms of group living, they are an excellent system in which to investigate the evolution of features that characterize sociality. While the phylogeny of the vespids has been scrutinized and often revised over the decades, it has been enduringly resolved based on morphological, behavioral, and molecular evidence that two subfamilies—the Vespinae and the Polistinae—are sister groups that contain most of the eusocial vespids (Carpenter 1982, 1991, 2003; Schmitz and Moritz 1998; Arévalo et al. 2004; Hines et al. 2007; Bank et al. 2017; Peters et al. 2017; Piekarski et al. 2018). The vespines are considered highly eusocial and typically have a morphologically distinct and unmated worker caste, whereas the polistines range from highly to primitively eusocial, with reproductively plastic workers that may or may not form a morphologically distinct caste (Hunt 2012, Piekarski et al. 2018). Another important aspect of the biology of the highly social vespids is how they initiate new colonies. Species within both the Vespinae and the Polistinae typically either found nests independently or through swarm founding (Jeanne 1991, Matsuura 1991). Across species, these modes of nest founding have been linked to the presence of two sternal exocrine glands in the highly social vespids.

Many vespines and polistines possess sternal exocrine glands known as the van der Vecht gland and Richards' gland, named after the wasp specialists who described their presence across numerous species (van der Vecht 1968, Richards 1971). These glands are not found in other wasps, bees, or ants, nor are they found in all vespine and polistine wasps. The presence of these glands has been examined across Vespinae + Polistinae (summarized by Downing 1991; Smith et al. 2001, 2002; Samacá et al. 2013; da Silva et al. 2021), although not for all species in this diverse clade, or always in fine detail. In surveys to date, the anatomy of the glands, when present, differs depending on species, caste, and sex (reviewed by Downing 1991, da Silva et al. 2021). For both glands, there have been efforts to reconcile their presence with a function that is shared across the behaviorally diverse social wasps that bear them.

When it is present, the van der Vecht gland is located on the sixth (terminal) metasomal sternite. In females, it is typically formed by two clusters of gland cells on either side of the midline at the anterior margin of the sternite, although some individuals have a single continuous band (Landolt and Akre 1979, reviewed by Downing 1991). The van der Vecht gland is a class 3 gland, meaning it is comprised of many bicellular units of secretory and duct cells (Noirot and Quennedey 1974, Billen and Morgan 1998, da Silva et al. 2021). The secretory cells synthesize pheromones that are released externally via

ducts that pass through the integument (Delfino et al. 1979), and often with external bristles or tufts of setae (van der Vecht 1968, Landolt and Akre 1979, Post and Jeanne 1980, Jeanne et al. 1983, Downing 1991, Martin 1992). The van der Vecht gland has been found in females of all vespines and the independent-founding polistines that have been examined to date (van der Vecht 1968, Landolt and Akre 1979, Post and Jeanne 1980, Jeanne et al. 1983, Martin 1992, Smith et al. 2001; reviewed by Downing 1991, da Silva et al. 2021). In contrast, the gland is absent in most of the swarm-founding Polistinae (Jeanne et al. 1983, Smith et al. 2001). Thus, it has been hypothesized that secretions from the van der Vecht glands help to protect unattended colonies from ant predation while young, workerless foundresses are away foraging (Jeanne 1970, Jeanne et al. 1983, Smith et al. 2001). Among the independent-founding polistines, van der Vecht gland secretions are repellent to ants, and females rub their gasters on their nests (Jeanne 1970; Hermans and Dirks 1974; Turillazzi and Ugolini 1979; Litte 1981; Post and Jeanne 1981; Kojima 1983, 1992; Keeping 1990; Dani et al. 1996, reviewed by Jeanne 1996). Queens also use van der Vecht secretions to exert dominance in some polistines (Dapporto et al. 2007). For swarm-founding polistines in which the van der Vecht gland is present, its secretions do not repel ants (London and Jeanne 2000) and the function of the gland remains unclear (da Silva et al. 2021). Compared to the polistines, the role of these secretions in the vespines is not as well documented. However, van der Vecht secretions from *Vespa affinis* L. and *V. tropica* L. do repel ants (Martin 1992) and a *V. analis* F. (all species: Hymenoptera: Vespidae) foundress was observed rubbing the tip of her gaster on her nest pedicel (Makino 2010).

The Richards' gland is located on the fifth (penultimate) metasomal sternite and is found in many but not all vespines and polistines (reviewed by Downing 1991, Smith et al. 2002, Samacá et al. 2013, da Silva et al. 2021). When present, it is often formed by a broad band of class 3 glandular units found along the anterior margin of the sternite (Heselhaus 1922, Richards 1971, Landolt and Akre 1979, Turillazzi 1979, Post and Jeanne 1980, Jeanne and Post 1982, Jeanne et al. 1983, Samacá et al. 2013, da Silva et al. 2015). Many cuticular modifications are also associated with this gland, including bristles or hairs, scales, grooves, and ridges (Jeanne and Post 1982, Jeanne et al. 1983). In general, the distribution of class 3 cells on the fifth metasomal sternite can be highly variable, so it has been proposed based on the study of polistines that the Richards' gland be restricted to only the class 3 cells at the base of the antecostal ridge and with scale-shaped modifications (Samacá et al. 2013). Early observations strongly suggested that secretions from this gland are used by some swarm-founding polistines to create chemical trails for swarm orientation (Naumann 1975, Jeanne 1981, Jeanne et al. 1983). However, swarm-founding behavior is not closely associated with the presence of the Richards' gland across Vespidae (Smith et al. 2002), so the function of the Richards' gland remains uncertain for most vespids (Samacá et al. 2013, da Silva et al. 2021). In *Vespa* L. (Hymenoptera: Vespidae), there is evidence that secretions from gynes act as a sex attractant (Wen et al. 2017) and are repellent to ants, like the van der Vecht gland (Martin 1992).

In this study, we explore the structure of the van der Vecht and Richards' glands in the giant hornet, *Vespa soror* du Buysson, and their use. The giant hornets—*V. soror* and its sister species *Vespa mandarinia* Smith (Hymenoptera: Vespidae) (Archer 1993, 1995; Perrard et al. 2013)—typically form large eusocial colonies that are founded annually by independent, overwintered queens that build partially enveloped nests underground (Matsuura and Sakagami 1973, Lee 2009). Giant hornet workers engage in group predation to meet their colony's nutritional demands by overwhelming colonies of

other species of eusocial wasps and honey bees, from which they take undefended brood as food for their larvae (Matsuura and Sakagami 1973, Matsuura 1984, Lee 2009, Mattila et al. 2020). Group predation by giant hornets requires the coordinated arrival of many hornet nestmates to the site of a group attack. It has been stated that scouting hornets recruit their nestmates to prey colonies using a marking pheromone secreted by the van der Vecht gland because they have been observed rubbing their gasters on target colonies and surrounding vegetation (Ono et al. 1995, Lee 2009, Mattila et al. 2020). Use of the gland in this way would be an innovation among the Vespidae. However, to date, no one has described the sternal glands of giant hornets. Furthermore, the proximate mechanisms by which nestmates get recruited from their home nest to the prey nest remain largely unexamined. Progress to date includes the identification of the ester 1-methylbutyl 3-methylbutanoate as a major component of both the van der Vecht and venom glands for *V. mandarinia* (Ono et al. 2003). Gland secretions for *V. soror* have not been characterized, although different studies have shown that presentation of this ester or extracts from the gastral tip of either giant hornet to sympatric *Vespa*, *Apis* L. (Hymenoptera: Apidae), and *Polistes* Latrille (Hymenoptera: Vespidae) prey species elicits strong defensive reactions (Ono et al. 1995, 2003; McClenaghan et al. 2019; Mattila et al. 2020). Despite these intriguing observations, there remains a need to better understand the sternal glands of giant hornets and their potential role in gastral rubbing for nestmate recruitment.

Within the vespines, the van der Vecht gland has been found in all species examined to date, although the Richards' gland is not always present (Heselhaus 1922; van der Vecht 1968; Landolt and Akre 1979; Smith et al. 2001, 2002). The genus *Vespa*, which includes 22 species worldwide (Smith-Pardo et al. 2020), has been largely ignored in these surveys. Both glands have been confirmed as present in *V. crabro* L. (Hymenoptera: Vespidae), *V. tropica*, and *V. affinis* only and the sternal brush of the van der Vecht gland of *V. analis* has been described (Heselhaus 1922, van der Vecht 1968, Landolt and Akre 1979, Martin 1992). Using light and electron microscopy, we confirmed the presence of both glands in *V. soror* and we examined their fine structure. The location of these glands makes them good candidates for contributing to a marking pheromone, and known responses to their secretory products are also suggestive. For instance, prey species respond defensively to extracts from the van der Vecht glands of giant hornets (Ono et al. 1995, 2003, Mattila et al. 2020). While the Richards' gland has not previously been considered to contribute to the marking pheromone of giant hornets, it is used to guide workers during trail formation by some polistine wasps (Naumann 1975, Jeanne 1981, Smith et al. 2002). Paired with morphological descriptions, we provide a detailed examination of the gastral rubbing behavior of hunting *V. soror* workers and consider the possibility that more than one exocrine gland is used by giant hornets as they recruit nestmates to initiate coordinated group attacks on their prey.

## Materials and Methods

### Sample Collection and Preservation

A sample of adult *V. soror* workers ( $n = 122$  specimens) was collected in the commune of Muong Leo, Sop Cop district, Son La province in northwestern Vietnam in 12 October 2020 (GPS coordinates: 20.836 N, 103.308 E). Foraging workers were sampled from the entrance of a colony that occupied an abandoned termite nest about one meter below ground. All workers were transported

to Hanoi, Vietnam, where they were preserved before shipment and eventual imaging at other locations.

A portion of the sampled workers had their terminal sternites immediately removed after field collection and preserved for sectioning and imaging of internal gland structure. To stabilize the cellular structure of sternal tissues, freshly killed hornets were dissected in a 2% glutaraldehyde fixative in a buffer containing 50 mM Na-cacodylate and 150 mM saccharose at pH 7.3. Micro-dissecting spring scissors (Roboz Surgical Instrument Co., Gaithersburg, MD) were used to carefully separate the terminal two (fifth and sixth) metasomal sternites from each hornet's body. The sternites were kept attached to each other to preserve the structure of connecting tissues. They were submerged in 2% glutaraldehyde fixative and refrigerated for 24 hr. The fixative was subsequently removed from the vials and replaced with a cold buffer to immobilize the tissues and prevent changes in pH. After 10 min, the buffer was removed and replaced with fresh buffer. All samples preserved in this way were then shipped to the University of Leuven (Belgium) for further processing and imaging of internal gland structure via light and electron (SEM and TEM) microscopy.

The intact bodies of the rest of the workers were preserved in 95% ethanol for external imaging. Because of the size of giant hornets, ethanol was injected directly into the abdomen and thorax of each freshly killed hornet. After one week, the specimens were re-injected with fresh ethanol and the ethanol was replaced in their storage containers, after which time all specimens were sufficiently preserved for shipment. They were sent to Wellesley College (USA) and Hosei University (Japan) for external imaging via light microscopy and SEM, respectively.

### Images of Internal Gland Structure

Before sectioning, sample tissues were dehydrated in a graded acetone series and embedded in Araldite (Huntsman International, The Woodlands, TX). Serial semithin sections (1  $\mu\text{m}$  thickness) were made with a diamond knife using a Leica EM UC6 ultramicrotome (Leica, Wetzlar, Germany). Sections were stained with methylene blue and thionin and viewed with an Olympus BX-51 light microscope (Olympus, Tokyo, Japan). Sections of the sternites were also examined via TEM with a Zeiss EM900 electron microscope (Zeiss, Oberkochen, Germany). These sections (70 nm) were double-stained with uranyl acetate and lead citrate before imaging.

The internal structure of van der Vecht gland tissues was also examined via SEM. To generate these images, the sixth sternite of a subset of specimens was dissected to remove nonglandular tissue. Some samples were also cut longitudinally across the cuticle of the sixth sternite in the location of one of the lateral glandular clusters. All prepared tissue was then dehydrated with a Balzers CPD 030 critical point dryer (BAL-TEC AG, Balzers, Liechtenstein) and coated with a thin gold layer using an SPI-Module Sputter Coater (SPI Supplies, West Chester, PA). The internal structure of the gland morphology was imaged with a Jeol JSM-6360 scanning microscope (JEOL Ltd., Tokyo, Japan).

From these images, we estimated the mean diameter for secretory cells of each gland. Cell diameter was measured at the widest point for cells in two semithin sections from different parts of the van der Vecht gland ( $n = 86$  cells) and from three semithin sections for the Richards' gland ( $n = 60$  cells).

### Images of External Gland Structure

The external features of the van der Vecht and Richards' glands were examined with SEM. Ethanol-preserved specimens were dissected under an SZ2-ILST stereo microscope (Olympus, Tokyo, Japan)

to remove the fifth and sixth sternites. Some of the sternites were cleaned with a fine paintbrush to remove debris; other sternites were left in their natural state. After air drying, the sternites were sputter-coated with gold by a Quick Coater SC-701S (Sanyu Electron Ltd., Tokyo, Japan). Scanning electron micrographs were taken using a JEOL-JCM-6000PLUS SEM microscope (JEOL Ltd., Tokyo, Japan). Pore diameter was determined for both glands from images of a *V. soror* worker ( $n = 30$  pores for the van der Vecht gland;  $n = 20$  pores for the Richards' gland). We also used these images to approximate the number of pores (and thus bicellular glandular units) for each gland. For the van der Vecht gland, we used images from two workers, from which we counted all pores in a lateral pore cluster and multiplied each value by two to give an estimated range in pore number for the entire gland per worker. We generated an estimated range in pore number for the Richards' gland by determining the number of pores in two SEM images taken from one worker of different regions of its gland. Both images spanned the band of pores from the gland's anterior to posterior boundaries. We averaged these two pore counts and then extrapolated this mean to the entire area of the gland, as determined for the single SEM-imaged specimen and using the mean gland area that was estimated for workers that were examined under a stereoscope.

For this latter group of workers, the fifth and sixth sternites were removed from 25 ethanol-preserved specimens and imaged using a SMZ-168 stereo microscope with an attached Moticam 2000 camera (Motic Instruments Inc., Richmond, Canada). These images were used to estimate the mean cuticle area per worker associated with features of each gland. The external area of the Richards' gland was determined by the boundaries of the band of pore openings at the anterior margin of the fifth sternite. The boundaries of the pore clusters for the van der Vecht gland were not discernable with a stereoscope because of overlapping setae, so instead we estimated the area of the hyaline region that contained the sternal brush (as a proxy for size of gland structures). Gland area was correlated with body size (Pearson correlation, SAS version 9.3, SAS Institute 2013), measured for each worker from their head to the apical margin of their fourth metasomal tergite using digital calipers (Marathon Watch Company Ltd., Vaughan, Canada).

### Observations of Gastral Rubbing in the Field

Videos of *V. soror* workers visiting *Apis cerana* F. (Hymenoptera: Apidae) colonies were made in a commercially managed apiary ( $n = 136$  colonies) in the commune of Ba Trai, Ba Vi District, Hanoi Province, Vietnam (GPS coordinates: 21.118 N, 105.335 E). Over three days (28–30 August 2013), eight observers were stationed throughout the apiary and each time one or more *V. soror* workers approached a hive, the visit was recorded using a Handycam HDR-PJ340 digital HD video camera (Sony Corporation, Tokyo, Japan), from as close to the start of the visit as possible until the last attacking worker departed from the hive. We observed both solitary and multiple-hornet attacks, but attacks were halted before they progressed to the slaughter phase (Matsuura and Sakagami 1973) to prevent colony loss. Care was taken to zoom in when necessary so that behaviors were discernible in the videos and predator-prey interactions were not disturbed by the proximity of human observers. These videos were subsequently reviewed to determine which ones captured *V. soror* workers rubbing their gasters on the hive they were visiting (see Results). For each visit in which gaster rubbing was observed, we determined the total number of hornet workers that were present and the number of those workers that rubbed or dragged any part of their gaster on the hive. It was not possible

to individually identify hornets, so we treated each occurrence of gastral rubbing as an independent replicate. For each gastral-rubbing worker, we estimated the number of bouts of rubbing per visit and the duration of each bout of rubbing following Kojima (1983), as well as the distance of the terminal end of the gaster at its closest and furthest point from the nearest margin of the hive entrance during each bout of rubbing.

On a different set of days (22–23 August 2013), we also characterized the gastral rubbing behavior of workers on nearby vegetation after they departed from hives in our study apiary, which was located in a grove of *Acacia mangium* Willd. (Fabales: Fabaceae) trees. Most of the trees had branches below 2 m removed to permit beekeeping activity and there was little vegetation at hive level. Thus, workers that rubbed their gasters on nearby vegetation did so up in the trees at heights of 2 m or higher, which made their behavior difficult to capture on video. For this reason, we documented their activities in real time, as well as the approximate height of each worker's position in the vegetation (at 0.5 m increments). If a worker flew upward, we noted whether she hovered or landed on vegetation and, if she landed, the duration of landing. For landed workers, we further noted whether they rubbed their gasters on vegetation or fanned their wings. From these observations, we determined the proportion of observations that included landing, gastral rubbing, or fanning. We compared the duration of landing and the height of landing sites between groups of workers that did or did not rub their gasters on vegetation ( $t$ -test, SAS version 9.3, SAS Institute 2013).

### Image Analysis

We used ImageJ image analysis software (National Institutes of Health, Bethesda, MD) to estimate size, distance, and area in the images that we generated. The scale was set for microscope images using their embedded scale bars; scale was set in still frames from videos using known widths of hive entrances, which were measured in the field for this purpose. Gland area was estimated in both stereoscope and SEM images using the measure function. To estimate pore number in an SEM image, we superimposed a grid on it and then used the multipoint tool to count pores within each grid square.

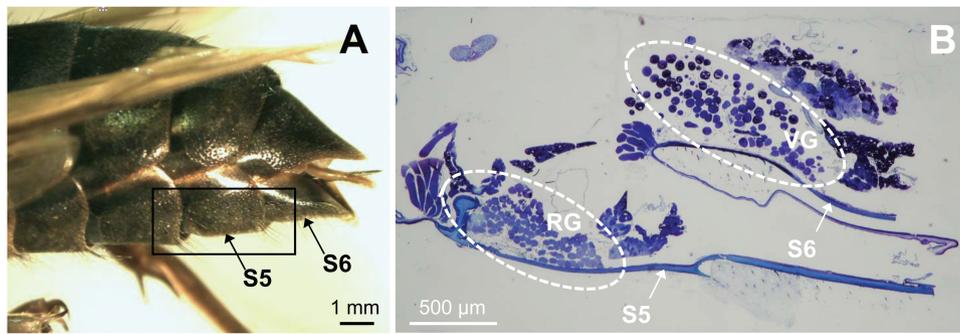
## Results

### Gland Morphology

All of the *V. soror* workers that we examined had well-developed van der Vecht and Richards' glands on their sixth (= terminal) and fifth (= penultimate) metasomal sternites, respectively (Fig. 1). Both glands were composed of class 3 bicellular units of secretory and duct cells. Structural details for each gland are provided below.

### Structure of the van der Vecht Gland

The van der Vecht gland was comprised of two lateral clusters of duct openings at the anterior margin of the sixth metasomal sternite, which were on either side of a well-developed sternal brush at the anterior midline (Fig. 2A and B). This configuration was visible on all specimens that were examined under a dissecting microscope ( $n = 25$  *V. soror* workers). The mean area of the medial anterior hyaline region, the boundaries of which encompassed the long setae (>1 mm) of the sternal brush, was  $3.17 \text{ mm}^2 \pm 0.4$  (SD) for these specimens, which correlated strongly with worker body size (Supp Fig. S1A [online only];  $r = 0.68$ ,  $P = 0.0002$ ). The hyaline area was generally concave relative to the plane of the rest of the sternite, which created a depression at the anterior midline in which most of the sternal



**Fig. 1.** A. External side view of the posterior abdomen of a *V. soror* worker. The black rectangle indicates the approximate area shown in the longitudinal section. B. Longitudinal semithin section lateral of the midline showing the position of the Richards' and van der Vecht glands (dashed white ovals) of the last two metasomal sternites. Arrows indicate sternites. Anterior is at left. RG = Richards' gland; S5 = metasomal sternite 5; S6 = metasomal sternite 6; VG = van der Vecht gland.

brush was held. Pores leading to duct cells were visible at the surface of the cuticle (Fig. 2C). The two workers we imaged with SEM had 2,255 and 2,535 pores, respectively, on one side of the sternite, which suggests that the van der Vecht gland is comprised of approximately 4,500–5,000 bicellular glandular units. Mean pore diameter at the sternite surface was estimated as  $3.8 \mu\text{m} \pm 0.5$  (SD) ( $n = 30$  pores). The area of one lateral cluster of duct openings per specimen was  $0.36 \text{ mm}^2$  and  $0.30 \text{ mm}^2$  for these two workers. Pore density was higher at the lateral margins of each cluster, where pores were interspersed with relatively short, bristle-like setae or no setae at all (Fig. 2D). Pore density was lower at the inner margins of the gland cluster, where pores were scattered among the longer setae of the outer margins of the sternal brush (Fig. 2E). With the exception of this transitional zone between lateral pore clusters and the medial sternal brush, there were no pore openings at the bases of the long setae of the sternal brush (Fig. 2F). The sternal brushes of most specimens were matted with material that was difficult to remove (Fig. 3A and B). The long setae at the posterior margin of the hyaline area were usually twisted together, a state that we observed in most specimens examined with SEM as well as under a stereoscope (Fig. 3). Across specimens, the hyaline region of the van der Vecht gland was lighter in color than the rest of the dark cuticle of the sternite (Supp Fig. S2A [online only]).

Semithin longitudinal and transverse sections through the van der Vecht gland illustrated that it was composed of loosely packed secretory cells (Fig. 4A–D). A transverse section at the anterior margin of the sternite confirmed that gland cells occurred in two lateral clusters and that secretory cells were absent at the midline (Fig. 4C), as suggested by the lack of duct openings in that area externally. The concave depression that was visible externally in the hyaline area of the sternal brush was clear in a midline longitudinal view as an upward bend of the anterior sixth sternite, which created a reservoir space between the sternite and the invaginated intersegmental membrane below it (Fig. 4A and C). Within this space, setae of the sternal brush were visible (Fig. 4B). The mean diameter of the secretory cells was estimated as  $50.8 \mu\text{m} \pm 10.9$  (SD) in the semithin sections ( $n = 86$  cells).

Ultrastructural observation revealed that the secretory cells each contained a polymorphic nucleus (Fig. 4E) and a branched end apparatus (Fig. 4F). The cytoplasm was dominated by rough endoplasmic reticulum (ER) (Fig. 4G). The duct cells displayed a small nucleus and a very reduced cytoplasm, and mainly contained a cuticle-lined duct with a constant internal diameter of  $1 \mu\text{m}$  (Fig. 4H) until they widened close to the pore openings at the sternal surface (Fig. 2C).

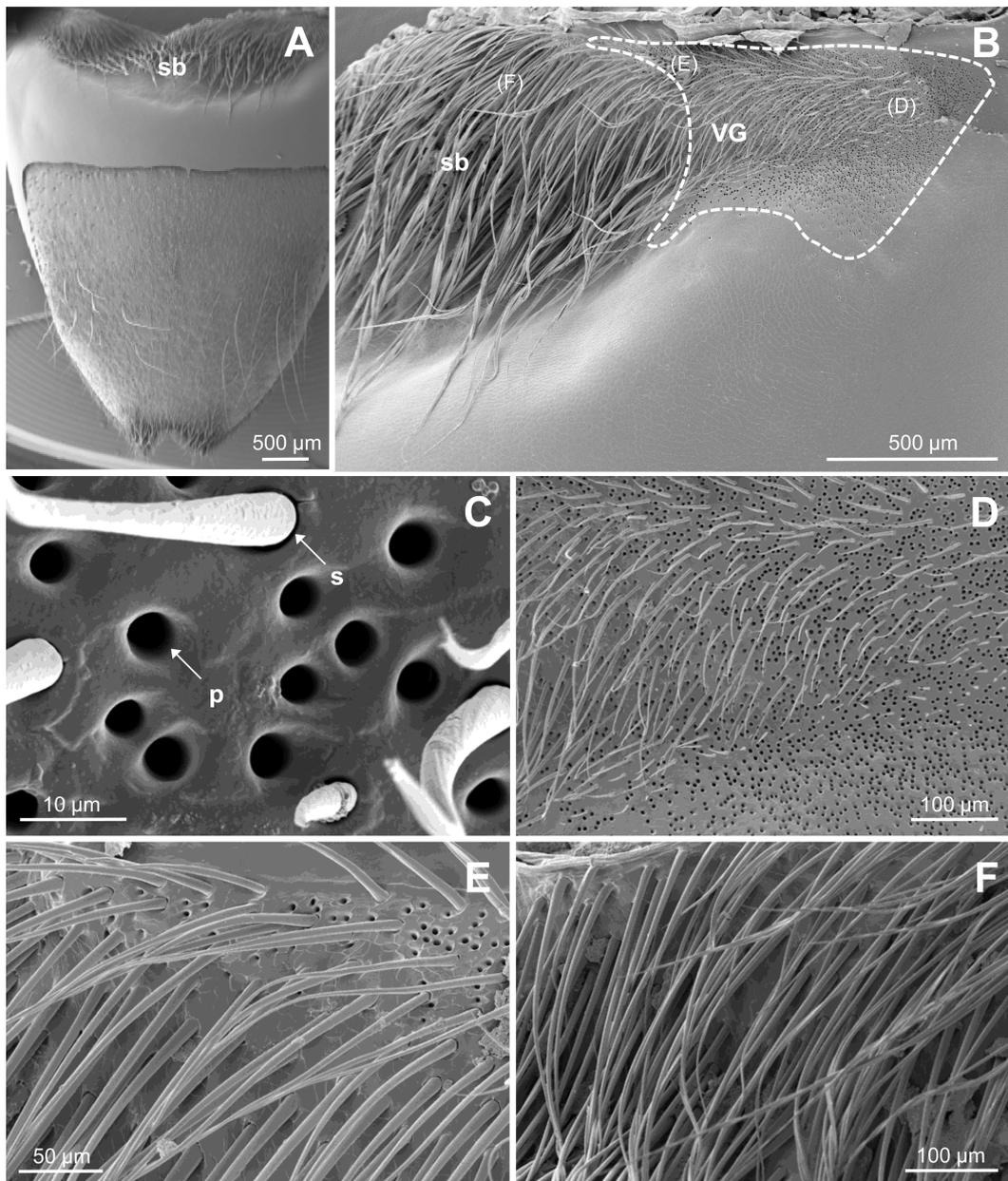
The two lateral clusters of gland cells were also conspicuous in SEM images of the internal side of the sixth sternite (Fig. 5A–C). The reservoir was visible between the fifth and sixth sternites, with stiff setae of the sternal brush positioned between them (Fig. 5B and D). Higher magnification gave clear details of the connected secretory cells and duct cells, as well as the passage of the latter through the cuticle to external cuticular pores (Fig. 5E and F).

#### Structure of the Richards' Gland

Externally, pore openings of a well-developed Richards' gland spanned the anterior margin of the fifth metasomal sternite in a band (Fig. 6A). The surface of the anterior cuticle was smooth, lacked substantial modification, and was lighter in color than the rest of the dark cuticle (Supp Fig. S2B [online only]). All of the sternites examined under a stereoscope had this configuration ( $n = 25$  *V. soror* workers). The area of the Richards' gland was estimated as  $1.24 \text{ mm}^2 \pm 0.20$  (SD) for these specimens, and  $1.92 \text{ mm}^2$  for the specimen imaged with SEM. Based on our estimate of the number of pore openings on this latter specimen and the two estimates of gland area, the Richards' gland is comprised of approximately 10,000–15,000 bicellular glandular units. Variation in gland area correlated well with body size (Supp Fig. S1B [online only];  $r = 0.60$ ,  $P = 0.001$ ). Some pores opened on the plane of the cuticle, while others were grouped into shallow pits (Fig. 6B and C). Mean pore diameter was estimated as  $4.9 \mu\text{m} \pm 0.4$  (SD) ( $n = 20$  pores).

Semithin sections of the fifth sternite confirmed that the band of dense external pores was matched in the same location internally by tightly packed layers of secretory and duct cell units (Fig. 7). Pore openings were posterior to the antecostal ridge, as were most of the secretory cells (Fig. 7A). A reservoir was visible in the space between the overlap of the anterior fifth sternite and the adjoining intersegmental membrane. Higher magnification images of the region of pore openings showed a layer of duct cells sandwiched between the secretory cells and the cuticle; the duct cells had a diameter of  $1 \mu\text{m}$  along most of their length, and passed through the cuticle to reach the enlarged external pores (Fig. 7B and C). Mean diameter of the secretory cells was  $51.7 \pm 8.0 \mu\text{m}$  (SD) ( $n = 60$  cells). When viewed in transverse section across the anterior region of the fifth sternite, the secretory cells of the Richards' gland filled the span of the sternite to its lateral edges (Fig. 7C), extending more distally than the lateral boundaries of the external pores (Fig. 6A).

Ultrastructural analysis showed that each secretory cell had a spherical to ovoid nucleus (Fig. 7D). The cytoplasm contained numerous mitochondria, secretory vesicles, and isolated strands of rough endoplasmic reticulum (Fig. 7); smooth ER was not clearly indicated. The end



**Fig. 2.** SEM images of the van der Vecht gland of a *V. soror* worker; anterior is top. A. Sixth metasomal sternite. B. Higher magnification of the lateral anterior margin showing the gland's sternal brush and a cluster of duct openings (outlined by the dashed white line); letters in parentheses indicate the location of Fig. 2D–F. C. Pore openings and setae. D–F. Pores are dense among the short lateral setae, but sparse or absent among the long setae of the sternal brush. p = pore; s = seta; sb = sternal brush; VG = van der Vecht gland.

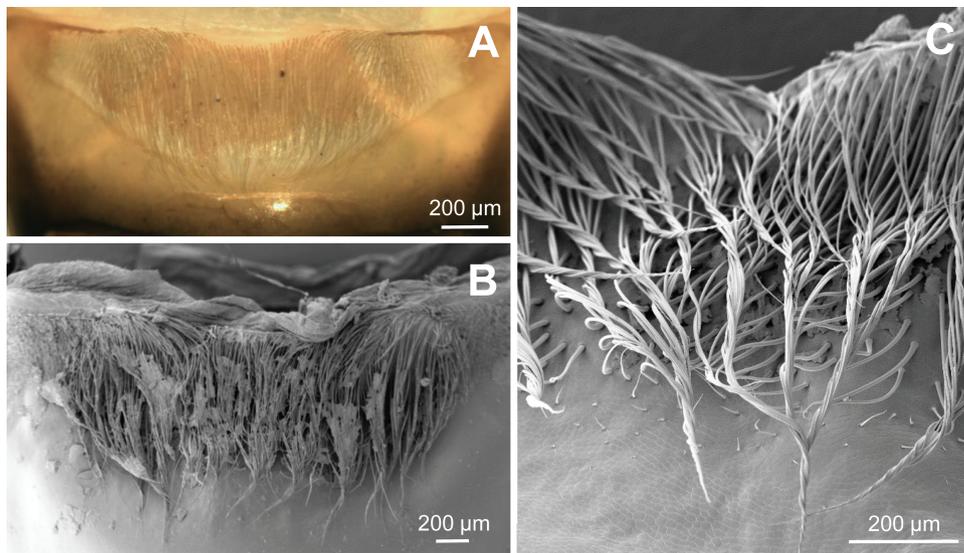
apparatus had an internal diameter of around  $0.5\ \mu\text{m}$  and contained several branches (Fig. 7F). Upon its connection to the duct cell, the diameter increased to  $1\ \mu\text{m}$  (Fig. 7G) and remained at this size until it approached the external opening site at the sternite's surface.

### Gastral Rubbing Behavior

We observed *V. soror* workers rubbing their gasters on hives and nearby vegetation on many occasions (Supp Videos S1–S4 [online only]). In 242 videos of visits to *A. cerana* hives, 21 videos captured at least one hornet on a hive front rubbing her gaster; 5 of these videos recorded two hornets visiting a hive at the same time and both rubbing their gasters on it ( $n = 26$  gaster-rubbing workers in total). The videos included a mix of single-hornet attacks and multiple-hornet attacks (10 vs. 11 videos, respectively).

Some colonies were rubbed only once, while others were rubbed repeatedly during consecutive visits by attacking hornets. *V. soror* workers rubbed their gasters on the hive fronts of a total of 12 different *A. cerana* colonies. Nine of these colonies were rubbed by a single worker during one visit (with up to three hornets present), but were not marked subsequently. In contrast, three colonies were visited on several occasions by gaster-rubbing workers (i.e., during 2, 4, or 6 videos of attacks by *V. soror* workers, respectively).

In general, if a *V. soror* worker rubbed her gaster on a hive during a visit, she did so during multiple bouts of rubbing, and usually close to the hive entrance. On average, if an individual worker performed this behavior, she spent a mean total of  $15 \pm 2\ \text{s}$  (range 3–55 s) per visit rubbing her gaster on the hive surface, summed over mean  $3 \pm 0.4$  bouts of rubbing (range 1–8 bouts of rubbing per visit). On



**Fig. 3.** Anterior margin (at top) of the sixth sternite of *V. soror* workers. A. Debris and dark secretion in the sternal brush of a specimen under a stereoscope. B. SEM image showing an uncleaned sternal brush and twisted setae. C. A cleaned specimen showing twisted setae.

average, the closest the tip of a worker's abdomen got to the nearest margin of the hive entrance during a bout of rubbing was mean  $2.4 \pm 0.5$  cm, and the farthest away was mean  $7.9 \pm 0.7$  cm. Most workers rubbed their gasters adjacent to entrances' margins or directly in front of them on landing boards. A few workers rubbed at the tops of hive fronts. Fig. 8 shows how rubbing activity was concentrated around the hive entrance of the colony that was visited most often by *V. soror* workers during the observation period. This attack was transitioning to the slaughter phase (Matsuura and Sakagami 1973); six workers were on the hive front and several of them were trying to chew the entrance open before we stopped them at the request of the beekeeper.

We also characterized the behavior of *V. soror* workers after they departed from hives in our study apiary (Supp Video S1 [online only]). On many occasions over two days, we observed workers flying upwards into vegetation above the hives after visiting them ( $n = 81$  observations). Most of the time, workers landed on leaves or branches, but sometimes they only hovered in a location before flying away (64 vs. 17 observations, respectively). About two thirds of workers that landed in the trees rubbed their gasters on vegetation (64.1%; 41 observations). Some workers fanned their wings while landed; these workers tended not to drag their gasters (8 out of 9 observations of fanning). Workers rubbed their gasters on vegetation at an approximate mean height of  $5.4 \pm 0.3$  m from the ground while landed for mean  $23 \pm 2$  s before flying away. These values did not differ from workers that did not rub their gasters while landed (mean duration landed:  $22.7 \pm 7$  s;  $t = 0.2$ ,  $df = 62$ ,  $P = 0.83$ ; mean height  $5.0 \pm 0.7$  m;  $t = 0.5$ ,  $df = 58$ ,  $P = 0.60$ ).

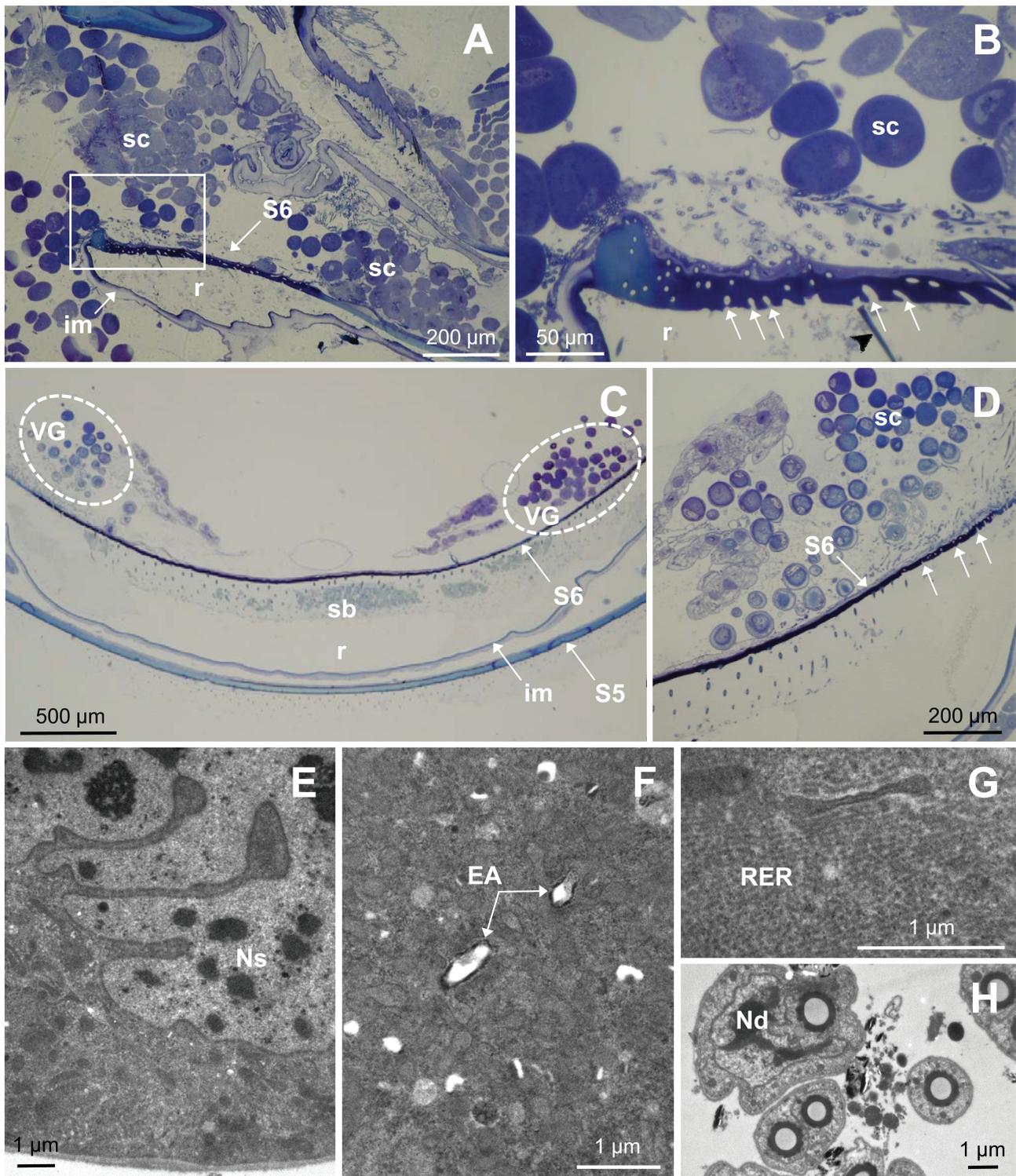
When rubbing, a worker swung her body back and forth while walking and pressing the ventral side of her gaster onto a hive surface (Supp Fig. S3A and Supp Videos S2–S4 [online only]). Sometimes this lateral swing was slight, but other times it was pronounced. It was usually not possible to discern which gastral sternites were pressed to the hive surface, but often the terminal segment was visibly extended. The extension revealed the smooth cuticle of the sixth tergite dorsally (the video's view), which likely also revealed the van der Vecht gland ventrally (Fig. 2A). In fact, the hyaline region of the sternal brush was visible in a video of a worker rubbing her gaster on abandoned equipment from an *A. cerana* colony (Supp Fig. S3B and

Supp Video S3 [online only]). Often a worker's extended position suggested that the Richards' gland was also exposed, although further work is necessary to confirm its involvement in rubbing.

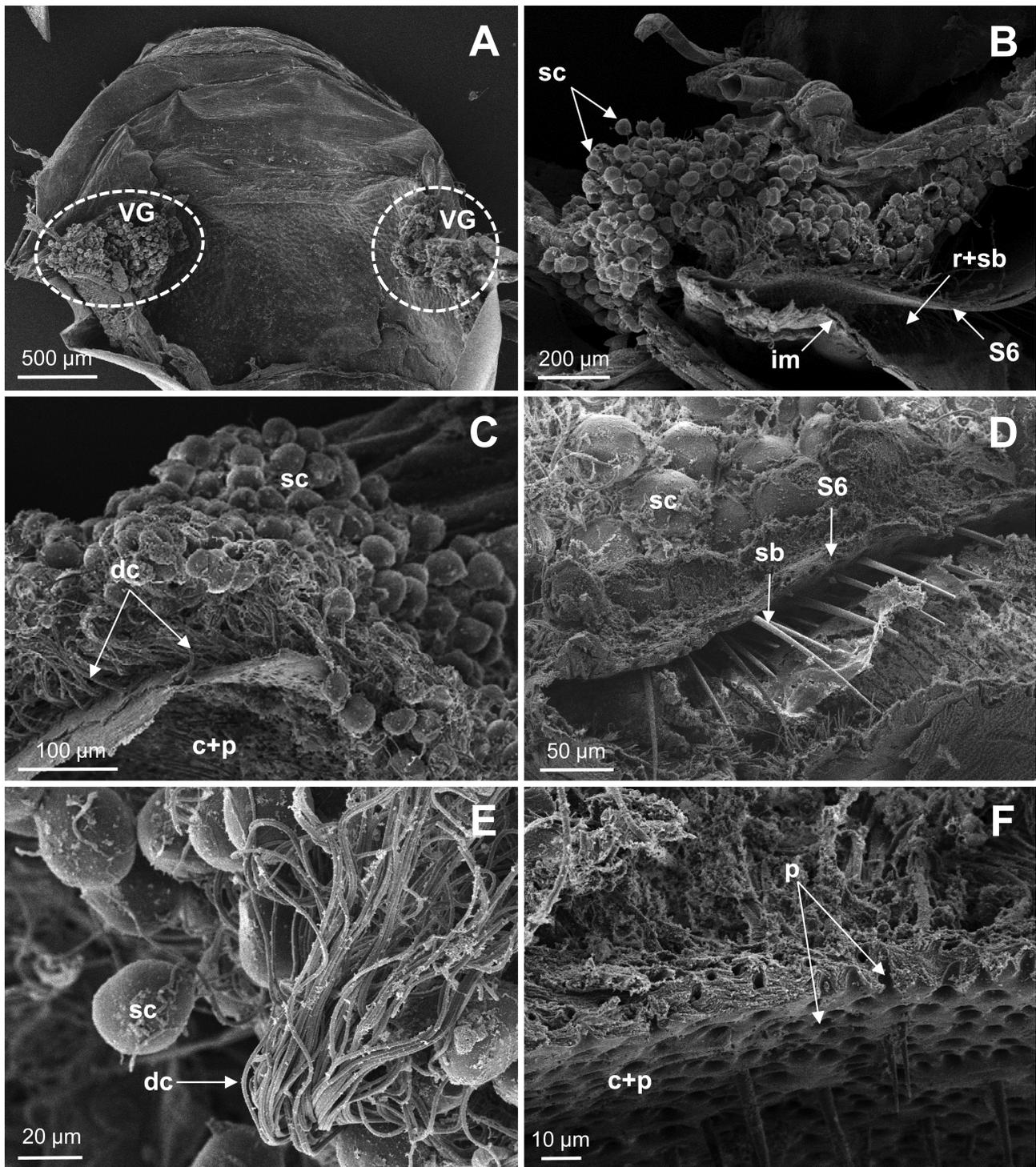
Finally, other exocrine glands may be involved in marking colonies or creating pheromone trails to guide nestmates to potential prey. In many instances, it appeared that *V. soror* workers extended a part of their sting apparatus beyond the tip of the gaster because something light colored (what appeared to be the third valvulae: Stetsun and Matushkina 2020) was visible beyond the dark cuticle of the gastral tip (Supp Video S4 [online only]). Some workers did this as they were dragging their gasters, while others did this between bouts of rubbing. In one instance, a worker extruded her stinger while landed on a hive with other hornets (Supp Video S5 [online only]). Workers in videos that included gastral rubbing often groomed their gasters and wings extensively with their hind legs or fanned while on hives and on vegetation. We did not observe *V. soror* workers rubbing other parts of their bodies directly on hive surfaces or vegetation.

## Discussion

Despite the fascinating spectacle of group predation by giant hornets and their tendency to target economically important social insects as prey (Matsuura 1988), little is known about how they recruit their nestmates to potential attack sites. Surprisingly, the features of glands that could support this activity have also been overlooked by decades of studies exploring the sternal glands of closely related taxa (reviewed by Downing 1991; Smith et al. 2001, 2002; da Silva et al. 2021). Our study describes in detail the morphology of two well-developed sternal glands of the giant hornet *Vespa soror* that, paired with field observations, provide insight into how hunting hornets use glandular secretions as a recruitment signal. All of the workers that we examined had conspicuous van der Vecht and Richards' glands, and gland features were similarly configured from worker to worker. Variation among workers in the size of both glands (i.e., cuticular area) was strongly correlated with differences in worker body size. Internally, both glands were class 3 in cellular structure, with paired units of secretory and duct cells (Noirot and Quennedy 1974). Externally, both glands were evident from aggregations of



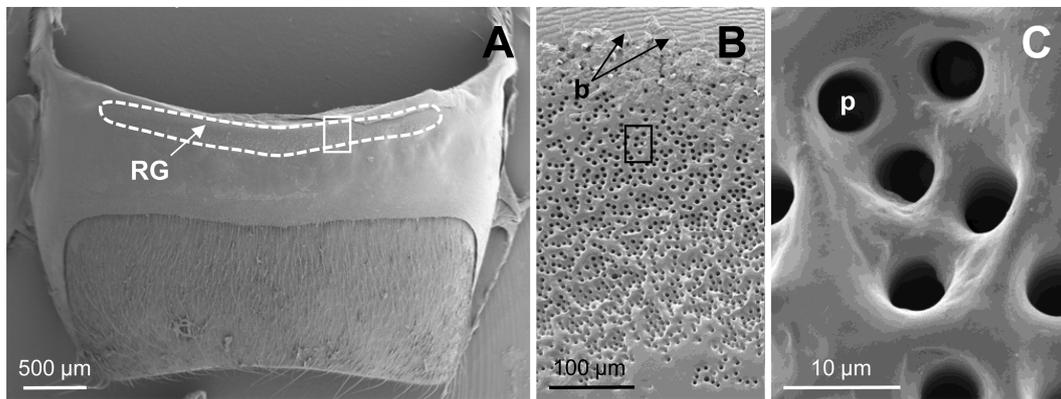
**Fig. 4.** Semithin sections of the van der Vecht gland of a *V. soror* worker. A. Longitudinal section lateral to the midline showing the secretory cells of the van der Vecht gland and the reservoir formed by the sixth sternite and the intersegmental membrane. B. Higher magnification of the white rectangle in A. Arrows indicate ducts opening at the surface of the cuticle; arrowhead indicates seta of the sternal brush. C. Transverse section at the anterior margin of the sixth sternite showing the two lateral clusters of gland cells (dashed white ovals) and the sternal brush in the reservoir between the sixth sternite and the intersegmental membrane + fifth sternite layer. D. Higher magnification of a transverse section; arrows indicate ducts opening at the cuticle. E–H. TEM images of cell ultrastructure. E. Polymorphic nucleus of a secretory cell. F. Branched end apparatus. G. Cytoplasm of secretory cells dominated by rough endoplasmic reticulum. H. Duct cells. EA = end apparatus; im = intersegmental membrane; Nd = nucleus of duct cell; Ns = nucleus of secretory cell; r = reservoir; RER = rough endoplasmic reticulum; sb = section through setae of sternal brush; sc = secretory cells; S5 = fifth sternite; S6 = sixth sternite; VG = cluster of van der Vecht glandular cells. Anterior at left (A, B); dorsal at top (C, D).



**Fig. 5.** SEM images of the structure of the van der Vecht gland of a *V. soror* worker, taken after making a longitudinal cut with a sharp razor blade so that internal and external features are visible simultaneously. A. Interior of the sixth sternite with two clusters of glandular cells (dashed white ovals); anterior at the top. B. Longitudinal view of a gland cluster above the reservoir formed by the sixth sternite and the intersegmental membrane, with the sternal brush visible; anterior at left. C. Secretory and duct cells (internal) above the cuticle and pores of the sixth sternite (external); dorsal at top. D. Stiff hairs of the sternal brush may aid in maintaining reservoir space. E. High magnification of secretory and duct cells. F. Longitudinal section through the sixth sternite, with duct cells (above) crossing the cuticle to external pores at the surface of the cuticle (below). c = cuticle; dc = duct cells; im = intersegmental membrane; r = reservoir; p = pores; sb = sternal brush; sc = secretory cells; S6 = sixth sternite; VG = van der Vecht gland.

thousands of pore openings at the surface of the cuticle, with reservoirs allowing secretions to accumulate for later application to a surface (Billen 2011). It remains to be determined what molecules these glands secrete, but ultrastructural examination suggested that

both produce proteins because of the presence of rough ER and lack of smooth ER (Delfino et al. 1979, 1982). Externally, *V. soror*'s van der Vecht and Richards' glands were differently structured from each other, but they each had many of the features that have been



**Fig. 6.** SEM images of the Richards' gland of a *V. soror* worker; anterior is top and squares show magnified series. A. Fifth metasomal sternite showing the band of duct openings of the Richards' gland (outlined by the dashed white line). B. Pore openings across a section of the band. C. Shallow pits contain clusters of pores. b = bristle; p = pore; RG = Richards' gland.

described for the sternal glands of the handful of other vespines and the many polistines that have been surveyed to date (reviewed by Downing 1991, da Silva 2021). Our detailed descriptions of the fine structure of the sternal glands of giant hornets add breadth and depth to information gleaned from drawings of external gland morphology for three other species in the genus *Vespa* (*V. tropica*, *V. affinis*, and *V. analis*: van der Vecht 1968, Martin 1992).

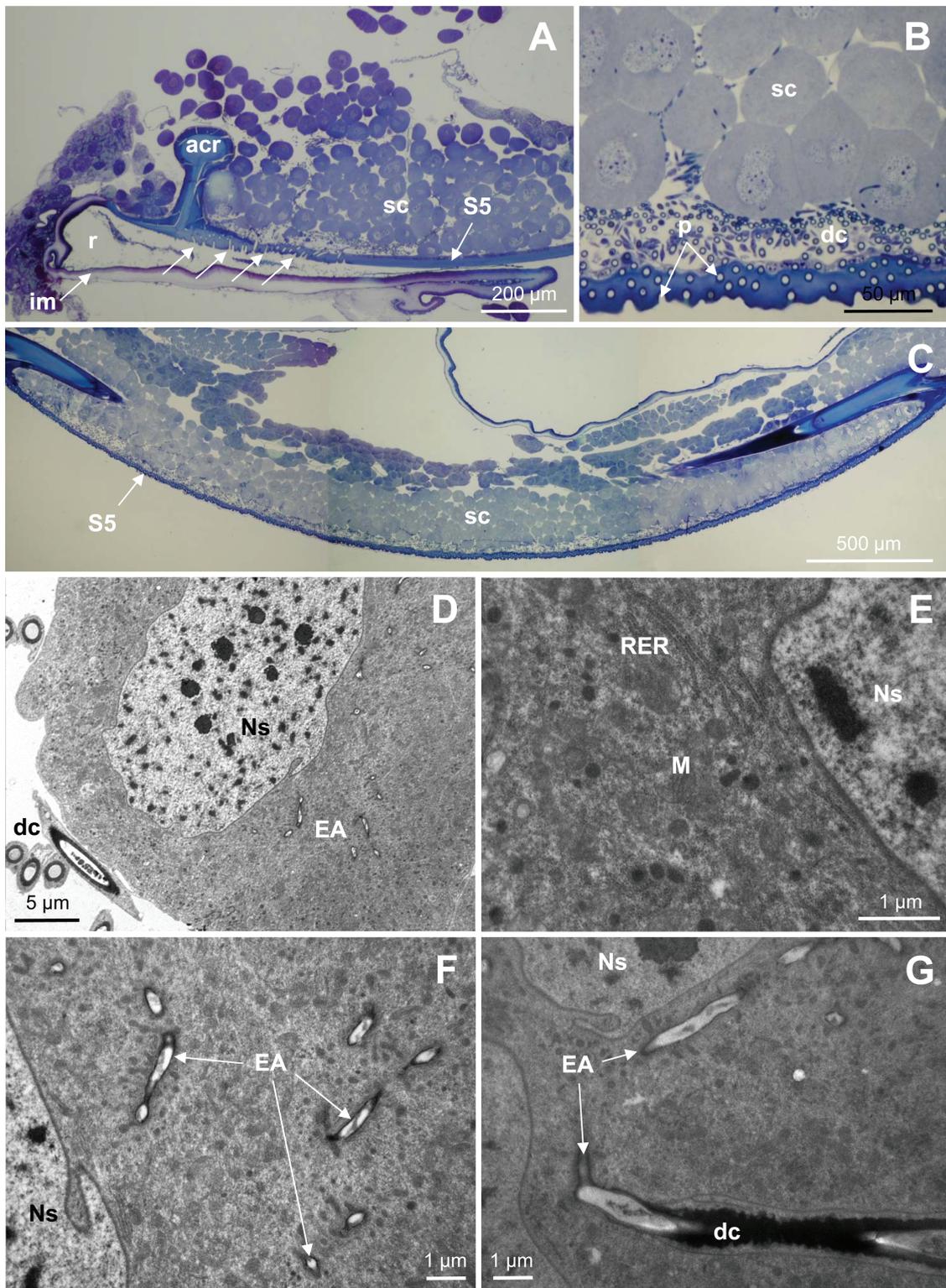
On the sixth metasomal sternite, the van der Vecht gland consists of two lateral clusters of pores that sandwich a large medial sternal brush at the anterior margin, matching the configuration observed in other vespines (van der Vecht 1968, Landolt and Akre 1979, Martin 1992). The sternal brush sits in a substantial depression in the cuticle, forming a reservoir around it by the overlap of the sixth sternite and the invaginated intersegmental membrane that attaches it to the fifth sternite (Billen 2011). This reservoir is expanded by the dorsal curve of the cuticle of the sixth sternite and its volume is likely maintained in part by stiff setae that are positioned to act as 'pillars'. Clusters of secretory cells and their associated duct cells were localized internally in lateral clusters that were visible as two groups of pores on the cuticle's exterior surface. The sternal brush was elaborate in all specimens, with the longest setae becoming intertwined and extending distally past the posterior margin of the hyaline area. Many sternal brush setae were matted with material that was probably a combination of gland secretions and debris picked up while rubbing, as has been reported in *Polistes* (Hermann and Dirks 1974). Workers often groomed themselves with their hind legs before and after gastral rubbing, which may help move gland products into the sternal brush or clean off debris picked up from surfaces. Grooming may also transfer compounds secreted by glands on other parts of their body to their gaster. The size of the sternal brush and length of setae are relatively greater in *V. soror* workers when compared to drawings of the external morphology of the same sternite for other hornets (van der Vecht 1968, Martin 1992), but detailed images for more *Vespa* species are necessary to confirm this impression.

The Richards' gland of *V. soror* is a well-defined band of dense pores that spans most of the anterior margin of the fifth metasomal sternite. The cuticle in this region is relatively featureless compared to the highly modified cuticle associated with the Richards' gland of many polistines (Jeanne and Post 1982, Jeanne et al. 1983, Samacá et al. 2013, da Silva et al. 2015), with the exception of some pores clustered in shallow pits, particularly in the posterior half of the band, and sparse, short setae at its anterior margin. Internally, the band was packed with multiple layers of secretory cells and a layer

of associated duct cells that drain posterior to the antecostal ridge. Thus, the structure of the Richards' gland of *V. soror* workers meets the restricted criteria for the Richards' gland defined by Samacá et al. (2013) for polistine wasps, except for a lack of highly modified cuticle around the pore openings.

If secretions from sternal glands provide a recruitment signal, then worker behavior is the means by which that signal gets communicated. Gastral rubbing has been noted for giant hornets and presumed necessary for recruitment (Ono et al. 1995, Lee 2009, Mattila et al. 2020), but it has not been described in detail. At our field site, gastral rubbing was uncommon; it was observed in only 9% of the videos we made of *V. soror* workers attacking *A. cerana* hives. Furthermore, when an individual worker rubbed her gaster during a visit, she did not spend a lot of time doing so—about 15 s on average over 3 bouts of rubbing—before she left. Of the colonies that were marked by a *V. soror* worker, most of them were marked during a single visit that did not progress to a group attack. However, brief occurrences of gastral rubbing by individuals probably add up to a well-reinforced signal over time as a colony becomes increasingly targeted by many gastral-rubbing workers. One colony received 26 bouts of gastral rubbing within three hours (Fig. 8) and attacking hornets had transitioned from hunting to attempting to breach the nest entrance. Secretions deposited during gastral rubbing likely dissipate over time, similar to how the repellent effect on ants of sternal gland secretions diminishes as hours pass (Turillazzi and Ugolini 1979, Kojima 1983, Martin 1992). However, the strength of a marking signal should increase if workers serially mark a colony within a window of a few hours, as happened in this instance. Marking has been characterized as a behavior that is performed by a lone hunting hornet (Ono et al. 1995), but our fieldwork showed that the majority of gastral rubs were performed when more than one hornet was present, sometimes by multiple workers during the same attack, and often after the first visit of the day, so it appears that the hornets' marking signal is continually reinforced by many workers during this early stage of nestmate recruitment to a prey colony.

An important question remains: which glands do *V. soror* workers utilize when they mark nests as potential targets for group predation? It is clear from our video footage that workers drag their van der Vecht glands on hive surfaces during gastral rubbing. Moreover, in still frames from the only video we have where the ventral side of a worker's gaster was visible during rubbing, the hyaline region with the sternal brush was visible (Supp Fig. S3 and



**Fig. 7.** Semithin sections of the Richards' gland of a *V. soror* worker. A. Longitudinal section showing the secretory cells of the Richards' gland and the reservoir formed by the fifth sternite and the intersegmental membrane; anterior at left. Arrows indicate a region with pore openings posterior to the antecostal ridge. B. High magnification longitudinal section of the region of pore openings, showing duct cells traversing the cuticle to pore openings. C. Composite transverse section of the fifth sternite showing densely packed secretory cells in a continuous layer behind the fifth sternite; dorsal at top. D–G. TEM images of cell ultrastructure, with junction between end apparatus and duct cell visible in G. acr = antecostal ridge; dc = duct cells; EA = end apparatus; im = intersegmental membrane; M = mitochondria; Ns = nucleus of secretory cell; p = pore; r = reservoir; RER = rough endoplasmic reticulum; sc = secretory cells; S5 = fifth sternite.

[Supp Video S3 \[online only\]](#)). The importance of the van der Vecht gland for recruitment is supported by the initiation of defensive responses by *A. cerana* colonies when they are exposed to its extracts

(Ono et al. 1995, Mattila et al. 2020). It is more difficult to confirm from our videos whether the Richards' gland was involved in gastral rubbing. The cuticle associated with both sternal glands is



**Fig. 8.** Gastral rubbing by *V. soror* workers was concentrated around hive entrances (marked by a black rectangle on hive). Each line (white, dashed) traces the tip of a *V. soror* worker's abdomen as she rubbed it on the hive front. This hive received the most gastral rubbing of any colony that we observed, with 9 hornets performing gastral rubbing in 6 videos of attacks recorded between 7–10 AM, for a total of 154 s over 26 bouts of rubbing. At the request of the beekeeper, we stopped the attack from proceeding by blocking the hive entrance, which multiple hornets were attempting to chew open.

notably lighter in color compared to the adjacent cuticle on the same sternites (Supp Fig. S2 [online only]), yet we could not see this light area on the fifth sternite in the single video where the sixth sternite's brush was visible. However, dorsal views during gastral rubbing by workers in the other attack videos gave the impression that the fifth sternite was extended and pressed onto the surface during rubbing. Martin (1992) found that secretions from both the van der Vecht and Richards' glands of *V. affinis* and *V. tropica* were repellent to ants, thus it is possible that the secretions from both glands could be selected in *V. soror* to support a shared function as well. The size of Richards' gland (2–3 times more glandular units than the van der Vecht gland) suggests that its secretory products are important. Thus, the Richards' gland remains an intriguing candidate for contributing to a marking signal in giant hornets, especially given its role in trail formation and nestmate recruitment in some swarm-founding polistines (Jeanne 1981, Smith et al. 2002) and the potential for its secretions to be transferred easily during gastral rubbing.

To further complicate this signaling scenario, part of the sting apparatus often protruded beyond the tip of the gaster as workers rubbed hive fronts. Moreover, many workers appeared to intentionally drag the tip or press it to hive surfaces during bouts of gastral rubbing (Supp Video S4 [online only]). During one multiple-hornet attack, we observed a stationary worker fully extrude her stinger and a substantial part of the sting apparatus, and the stinger shaft looked like it was glistening while being pulsed (Supp Video S5 [online only]). All female eusocial vespids examined to date have Dufour's and venom glands (Landolt and Akre 1979), the ducts of which open separately into the sting bulb or vaginal wall in *Vespa* and their relatives (Billen 1987, 2006; Silveira and Caetano 1993; Stetsun and Matushkina 2020). Both are candidate glands if secretions from the genital chamber at the tip of the gaster are involved in nestmate recruitment. While the function of the Dufour's gland remains unclear in general for Vespidae, it has been implicated in nestmate communication in other social contexts (reviewed by Downing 1991, Mitra 2013). Little is known about the glandular products of giant hornets specifically. However, the main biologically active volatiles in the venom of *V. mandarinia* have been identified and one component, 1-methylbutyl 3-methylbutanoate, is also a major component

of the van der Vecht gland (Ono et al. 2003). Exposure to chemical extracts from the venom gland causes *V. mandarinia* workers to rush toward their source when they are placed outside nest entrances, and the hornets' reaction was strongest in response to a mix of extracts that included components from both glands (Ono et al. 2003). In a different context or with a different mix of exocrine products, this defensive response could be modulated to generate an aggressive assembly of attacking hornets at the nests of their prey. A better understanding the chemical components of the marking signal is necessary to trace it to source exocrine glands, although this effort could be challenging if signal components are produced by more than one gland or are found in nonglandular tissue (Oldham et al. 1994, Dani et al. 2003, Ono et al. 2003).

Gastral rubbing by workers on target nests is likely one of the first steps for organizing a group attack by giant hornets. Currently, we know little about how worker activities at the nests of giant hornets further support the coordination of these attacks. Matsuura and Yamane (1990) briefly mention that observations of *V. mandarinia* nests have not revealed behaviors that could inform nestmates about the location of food, but rigorous studies have not been done. Signaling systems to communicate the location of food have evolved in all eusocial insect lineages except the vespids (Jeanne 1991, 1996; Bruschini et al. 2010), for which mechanisms for direct nestmate recruitment are absent where they have been sought (Jeanne et al. 1995, Overmyer and Jeanne 1998, Raveret Richter 2000, Kim et al. 2007). Viewed in this light, our field observations of workers rubbing their gasters on hives and nearby vegetation is exciting. They confirm that, at the very least, workers produce signals near prey colonies that could guide nestmates to food. Whether this is part of a more extensive trail is yet to be determined. Most giant hornets forage in about a 1–2 km radius around their nests, with nest visitation rates the highest for social insect prey that are less than 1 km away (Matsuura and Yamane 1990). Thus, it is possible that foraging hornets could encounter nestmates engaged in an attack by chance (especially when prey colonies are concentrated in an apiary) and then join that effort without following a trail or responding to other recruitment signals. However, the rapid build-up of workers that we observed at colonies with heavy gastral rubbing suggests that signaling plays a role in recruitment. Future field work should focus on 'connecting the dots' between locations of gastral rubbing on prey nests and nearby vegetation and other potential marking sites on the way back to the hornets' home. Use of multiple signaling sites is typical in other flying eusocial insects that create scent trails to recruit nestmates to food (e.g., stingless bees: Barth et al. 2008) or nest sites (e.g., polistines: Jeanne 1981, Litte 1981). Conversely, some social wasps use glandular secretions as short-distance assembly signals or orientation cues without maintaining scent trails (West-Eberhard 1982, O'Donnell 1992, Jeanne 1996) and a growing body of evidence suggests that many stingless bees deposit recruitment marks primarily around the food source (reviewed by Jarau 2009). Use of exocrine secretions by gastral-rubbing giant hornets lies somewhere on this spectrum.

We have shed some light on the connection between exocrine glands, gastral rubbing behavior, and potential mechanisms of the recruitment of nestmates to food by the giant hornet, *V. soror*. Workers have well-developed van der Vecht and Richards' glands, a minority of hunting hornets engage in gastral rubbing on and around prey nests, and this brief but vigorous behavior likely produces a recruitment signal that is collectively reinforced by additional workers over subsequent visits to an attack site. It remains unclear which exocrine glands contribute to this signal. Our behavioral observations confirm that the van der Vecht gland is used during marking, but that

the Richards', Dufour's, and venom glands could be involved as well. Despite many gaps remaining in our understanding of chemical communication among social insects, there are emerging patterns in the substantial body of literature about exocrine glands, their secretions, and recruitment to food in the eusocial Hymenoptera that should be considered here. Firstly, recruitment signals produced by exocrine glands are usually complex, with several glands contributing different types of chemicals (Hölldobler 1995, Barth et al. 2008, Morgan 2009, Cerdá et al. 2014). It is unlikely that only one gland is involved in producing the signals that are used by giant hornets to communicate during group predation. Secondly, an exocrine gland that is found among several eusocial taxa can be used by its bearers for many different purposes. For instance, all females of the eusocial aculeates have a Dufour's gland, yet the nature and functionality of its secretions vary tremendously across taxa (Mittra 2013), including playing a role in trail recruitment in ants and nest marking in bees (Hefetz 1987, Cerdá et al. 2014). It is probably overly simplistic to attribute a single 'universal' function to an exocrine gland or a singular compound it secretes (Smith et al. 2002); we should expect the discovery of innovative uses of the products of exocrine glands, even among closely related species. Finally, effective recruitment systems characterize the most ecologically dominant taxa of eusocial insects (Jeanne 1991, Wyatt 2003, Jarau 2009, Wilson and Hölldobler 2005), so this level of foraging organization is not unexpected for apex predators such as the group-hunting giant hornets.

## Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

Supp Fig. S1. Area of the hyaline region of the van der Vecht gland (A) and pores of the Richards' gland (B) both correlated significantly with worker body length, measured from the head to the apical margin of the fourth metasomal tergite.

Supp Fig. S2. The cuticle associated with glands at the anterior margin of each sternite (dashed white oval) is lighter in color than the surrounding darker cuticle of the rest of the sternite. A. Sixth sternite with the van der Vecht gland. B. Fifth sternite with the Richards' gland. Anterior is at the top.

Supp Fig. S3. Still frames from [Supp Video S3 \[online only\]](#) of a *V. soror* worker rubbing her gaster on a wooden frame of an abandoned comb from an *A. cerana* colony. A. Pressed and elongated position of the gaster during rubbing; black arrow shows the extension of the posterior tergites. B. White arrow highlights the ventral hyaline area of the sixth sternite that holds the sternal brush of the van der Vecht gland.

Supp Video S1. Three bouts of gastral rubbing by a *V. soror* worker recorded immediately after she left an *A. cerana* hive.

Supp Video S2. A *V. soror* worker rubbing her gaster at the entrance of an *A. cerana* hive. Posterior segments of the gaster are extended during rubbing.

Supp Video S3. A *V. soror* worker rubbing her gaster on the wooden frame of abandoned comb from an *A. cerana* colony. The hyaline region of the van der Vecht gland is briefly visible ventrally (as shown in [Supp Fig. 3 \[online only\]](#)).

Supp Video S4. A *V. soror* worker rubbing her gaster at the entrance of an *A. cerana* hive. Part of the sting apparatus is briefly visible during rubbing.

Supp Video S5. A multiple-hornet attack on *A. cerana* colony. Note the first *V. soror* worker on the left is fanning, the second stationary worker on the left extrudes her stinger, and two workers on the right try to widen the hive entrance by chewing it open.

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