



# Translucent cuticle and setiferous patches in Megasilidae (Hymenoptera, Ceraphronoidea)

Carolyn Trietsch<sup>1</sup>, István Mikó<sup>1</sup>, Jonah M. Ulmer<sup>1</sup>, Andrew R. Deans<sup>1</sup>

<sup>1</sup> Frost Entomological Museum, Department of Entomology, 501 ASI Building, The Pennsylvania State University, University Park, PA, 16802 USA

Corresponding author: Carolyn Trietsch ([cut162@psu.edu](mailto:cut162@psu.edu))

Academic editor: M. Yoder | Received 15 May 2017 | Accepted 16 August 2017 | Published 30 October 2017

<http://zoobank.org/D3E4FFEC-C9DD-4D5F-B0CC-A2B79ACA511A>

**Citation:** Trietsch C, Mikó I, Ulmer JM, Deans AR (2017) Translucent cuticle and setiferous patches in Megasilidae (Hymenoptera, Ceraphronoidea). Journal of Hymenoptera Research 60: 135–156. <https://doi.org/10.3897/jhr.60.13692>

## Abstract

All Ceraphronoidea have metasomal patches of translucent cuticle and setae that have never been investigated before, despite their potential behavioral and phylogenetic relevance. To understand the internal and external morphology of these structures, specimens were examined using a broad array of histology-based methods, including transmission electron microscopy (TEM), scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM) and serial block-face scanning electron microscopy (SBFSEM). For the first time, the setiferous patches are shown to be associated with exocrine glands in Ceraphronoidea. The proposed glandular function is the secretion of pheromones, with the setae above the pore openings serving as a surface for evaporation. The translucent cuticle is morphologically distinct from the setiferous patches; structures resembling lamellar bodies were found underneath the translucent cuticle, and may be associated with photoreceptors or endocrine glands. The locations of translucent cuticle on the metasoma are unique to different families and genera within Ceraphronoidea, and could be useful for inferring phylogenetic relationships. The character distribution suggests that the genera *Trassedia* and *Masner* are more closely related to Ceraphronidae than Megasilidae. We found similar structures containing translucent cuticle in Orussidae and Ichneumonoidea, indicating that these structures are potentially a rich character system for future phylogenetic analysis in Hymenoptera.

## Keywords

*Megaspilus*, *Conostigmus*, *Dendrocerus*, Lagynodinae, felt field, felt line, setal patch, translucent patch, thyridium, gastrocoelus

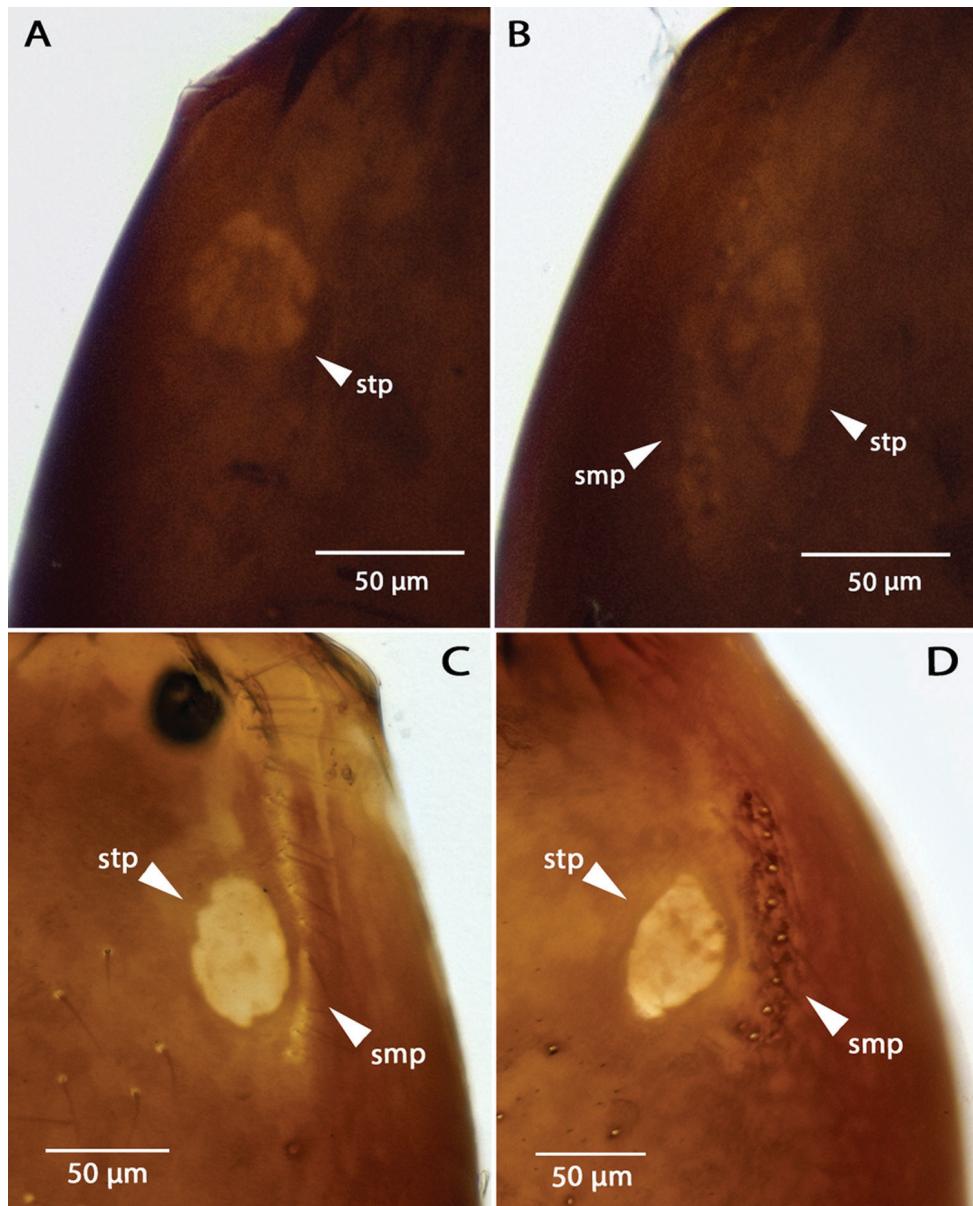
## Introduction

Ceraphronoidea is a small but widespread superfamily of parasitoid wasps that contains approximately 600 species and is comprised of two families, Megaspilidae and Ceraphronidae (Johnson and Musetti 2004). Although ceraphronoids are commonly collected (Martinez de Murgia et al. 2001; Mikó et al. 2013; Schmitt 2004) and include species that are agriculturally important (Boenisch et al. 1997; Chow and Mackauer 1999; Ferrière 1933; Kamarudin et al. 1996; Ortiz-Martínez and Laverdero 2017; Polaszek et al. 1994) the taxon is full of phylogenetic uncertainties. Even the relationship of Ceraphronoidea to other Hymenoptera remains unclear, although the superfamily is robustly monophyletic (Mikó et al. 2013). Different molecular analyses have grouped Ceraphronoidea with Stephanoidea, Ichneumonoidea, Melyroidea, or with Ichneumonoidea and Proctotrupomorpha (Klopfstein et al. 2013; Mao et al. 2014; Peters et al. 2017; Sharkey 2007; Sharkey et al. 2012). Contrary to the belief that ceraphronoids are too small for morphological characters to be of phylogenetic use (Klopfstein et al. 2013), the group contains taxa with morphological structures that may serve as characters to corroborate both the phylogenetic relationships among members within the superfamily.

On the metasoma of all ceraphronoid wasps, there are pairs of translucent patches of cuticle on the syntergite and synsternite, referred to as the syntergal and synsternal translucent patches (stp) (Mikó and Deans 2009) (Fig. 1). These translucent patches may be similar to the smooth patches of cuticle found on the metasoma of in Orussidae and Xiphydriidae (Vilhelmsen 2003), the gastrocoelus and thyridium of Ichneumonidae and Proctotrupidae, and the pseudothyridium which occurs widely across Hymenoptera (Liu et al. 2006; Townes 1969). In addition to these translucent patches, all Ceraphronoidea possess patches of setae on the synsternite near the synsternal translucent patches, known as the synsternal setiferous patches (smp) (Mikó and Deans 2009) (Fig. 1). These setiferous patches are only found on the ventral surface of the metasoma and appear similar to the felt lines and felt fields on the metasoma of other Hymenoptera, including Mutillidae (Debolt 1973) and Platygastroidea (Masner and Huggert 1989).

Even though translucent and setiferous patches have been observed in several hymenopteran taxa, little work has been done to investigate their morphology and potential functions. The translucent cuticle in the gastrocoelus, thyridium and pseudothyridium has never been studied before, even though differences in the thyridium have been used to distinguish between proctotrupid species (Liu et al. 2006). Translucent cuticle is found in many different insects and is associated with different functions, from light dispersal in fireflies (Coleoptera: Lampyridae) (Kim et al. 2012) to glandular activity in giant silk moths (Lepidoptera: Saturniidae).

More research has been done to investigate the structure and function of setiferous patches found in other Hymenoptera. In Mutillidae, there is a “felt line organ” underneath the felt lines that appears to function as an exocrine gland (Deb Bolt 1973).



**Figure 1.** Brightfield images of syntergal and synsternal translucent patches and synsternal setiferous patches in different species of *Conostigmus* (Hymenoptera: Megaspilidae), viewed externally. **A** Dorsal surface (syntergite) within a *C. bipunctatus* Kieffer, 1907 (Hymenoptera: Megaspilidae) specimen (identifier: IM 1751) **B** Ventral surface (synsternite) within the same *C. bipunctatus* specimen **C** Ventral surface of *Conostigmus* sp. C7A (identifier: CLEV 22741) **D** Ventral surface of *Conostigmus* sp. C7B (identifier: PSUC\_FEM 83781) Abbreviations: smp = synsternal setiferous patch; stp = syntergal/synsternal translucent patch. The species notations given are not issued for purposes of zoological nomenclature, and are not published within the meaning of the International Code of Zoological Nomenclature.

Debolt (1973) described ducts passing from gland cells in the felt line organ to cuticular pores located directly underneath the felt fields. Glandular pores and openings have also been observed underneath patches of setae in Megachilidae (Noirot and Quennedey 1974), Braconidae (Buckingham and Sharkey 1988) and Platygastroidea (Mikó et al. 2010). It has been proposed that setae might increase the surface area for the diffusion of glandular products secreted from these pores, such as pheromones (Buckingham and Sharkey 1988; Debolt 1973; Mikó et al. 2007, 2010; Noirot and Quennedey 1974; Quicke and Falco 1998). Given that pheromones play a wide variety of important ecological, behavioral and physiological functions within insects, understanding these structures could have important implications for species recognition, sexual selection, and other forms of chemically-mediated communication and behavior within Hymenoptera (Howard and Blomquist 2005).

To understand the morphology of the translucent and setiferous patches in Ceraphronoidea, we dissected and imaged specimens with brightfield microscopy, scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). Orussid and ichneumonid specimens were also dissected and imaged with brightfield microscopy for comparison. We also utilized histological methods; we used transmission electron microscopy (TEM) to investigate the cuticle and underlying structures, and serial block-face scanning electron microscopy (SBFSEM) to build three-dimensional representations of these structures. SBFSEM is a novel technology that has only recently been applied to studying arthropod physiology, but it is a promising approach for studying external and internal morphology (Büsse et al. 2016; Friedrich et al. 2014; Lipke et al. 2014). This study comprises the first in-depth investigation of the translucent patches, setiferous patches and underlying structures within Ceraphronoidea.

## Methods

Pinned and point-mounted Orussidae, Ceraphronoidea and Ichneumonoidea specimens were obtained from the Frost Entomological Museum (PSUC), the C. A. Triplehorn Insect Collection (OSUC), the American Museum of Natural History (AMNH), the North Carolina State University Insect Museum (NCSU), and the Wisconsin Insect Research Collection (WIRC). Live specimens for histology were collected with sweep nets and aspirators from local field sites around State College, Pennsylvania, USA. A list of the specimens used in this study and associated data is available in Suppl. material 1.

All specimen observations and dissections were done under an Olympus SZX16 stereomicroscope with an Olympus SDF PL APO 1X PF objective (115X) and an Olympus SDF PL APO 2X PFC objective (230X magnification). Point-mounted specimens were prepared for dissection by incubating them at room temperature in 20–25% KOH for 24 hours, acetic acid for 24 hours, and then distilled water for one hour. Afterwards, specimens were placed on individual concave slides in glycerin for dissection and storage. Dissections were done in glycerin with #2 insect pins and

#5 forceps. Brightfield images were taken using an Olympus DP71 digital camera attached to an Olympus ZX41 compound microscope. Images were then aligned and stacked using Zerene Stacker Version 1.04 Build T201404082055 (see protocol in Trietsch et al. 2015). Adobe Photoshop elements Version 3.1 was used to add scale bars to images and create figures.

For CLSM, metasomata were removed from point-mounted megaspilid specimens and either put directly into glycerin, or incubated at room temperature in 35% hydrogen peroxide for 48 hours before being put in glycerin. The purpose of this incubation was to bleach melanin-rich structures, which can interfere with autofluorescence. All metasomata were dissected in glycerin, mounted between 1.5 mm thick, 24×50 mm cover glasses and then imaged using an Olympus FV10i confocal laser scanning microscope. Auto-fluorescence of the structures was collected between 470 and 670 nm with three channels assigned contrasting pseudocolors (420–520nm, blue; 490–520nm, green; and 570–670nm, red). Images were processed in ImageJ (Version 2.0.0-rc-54/1.51g, Build 26f53ffab) (Schindelin et al. 2015) using FIJI (Schindelin et al. 2012).

For TEM, live megaspilid specimens were dissected in cacodylate buffer, fixed with glutaraldehyde, stained with osmium tetroxide and uranyl acetate, dehydrated through an ethanol series, and embedded in eponite (protocol available at <https://doi.org/10.6084/m9.figshare.4993793>). Blocks were trimmed and sectioned using a Leica UCT ultramicrotome. Sections were collected on slot and mesh grids and then double-stained with lead citrate and uranyl acetate. Sections were imaged with a JEOL 1200 TEM.

Live specimens for SBFSEM were also dissected in cacodylate buffer, fixed in glutaraldehyde, and then stained with osmium tetroxide, potassium ferrocyanide, thiocarbohydrazide (TCH) solution, uranyl acetate, and lead aspartate. Specimens were then dehydrated through an ethanol series and embedded in eponite (Protocol available at <https://doi.org/10.6084/m9.figshare.4993796.v1>), modified from Deerinck et al. (2010). Blocks were trimmed and sectioned using a Leica UCT ultramicrotome, then mounted into a Zeiss SIGMA VP-FESEM with a Gatan 3View2 accessory for sectioning and imaging. Data was processed in Avizo (Version 9.1.1). The images were aligned and cropped in ImageJ (Version 2.0.0) then imported into Avizo (Version 9.1.1). The images were stacked and volume rendered, then each unique morphological component was marked as an individual label field and modeled through manual outlining and interpolation. The generated surface model was smoothed and the polygon points were simplified to make the file more manageable. The images were then converted into a gif and respective jpeg images that allowed cross-sectional viewing of the models (Mikó et al. in prep). For SEM, metasomata were mounted on carbon tape on top of an aluminum stud. Half of the specimens used were coated in iridium, while half remained uncoated as a control. SEM images were taken on an FEI Quanta 200 Environmental SEM and processed in Aztec (version 3.1 SP1, Oxford Instruments).

Anatomical terms follow the Hymenoptera Anatomy Ontology (Yoder et al. 2010). All specimen data and images of specimens were compiled in the MX database

(<http://purl.oclc.org/NET/mx-database>). All figures, tables, media files, three dimensional models, protocols and supplementary files are available on figshare at [https://figshare.com/projects/Translucent\\_cuticle\\_and\\_setiferous\\_patches\\_in\\_Megaspilidae\\_Hymenoptera\\_Ceraphronoidea\\_21395](https://figshare.com/projects/Translucent_cuticle_and_setiferous_patches_in_Megaspilidae_Hymenoptera_Ceraphronoidea_21395). The specimen data included in Supplementary file 1 was published using the Integrated Publishing Toolkit (<https://www.gbif.org/news/82852/new-darwin-core-spreadsheet-templates-simplify-data-preparation-and-publishing>) and made available on GBIF and figshare (<https://doi.org/10.6084/m9.figshare.5325574.v1>).

## Results

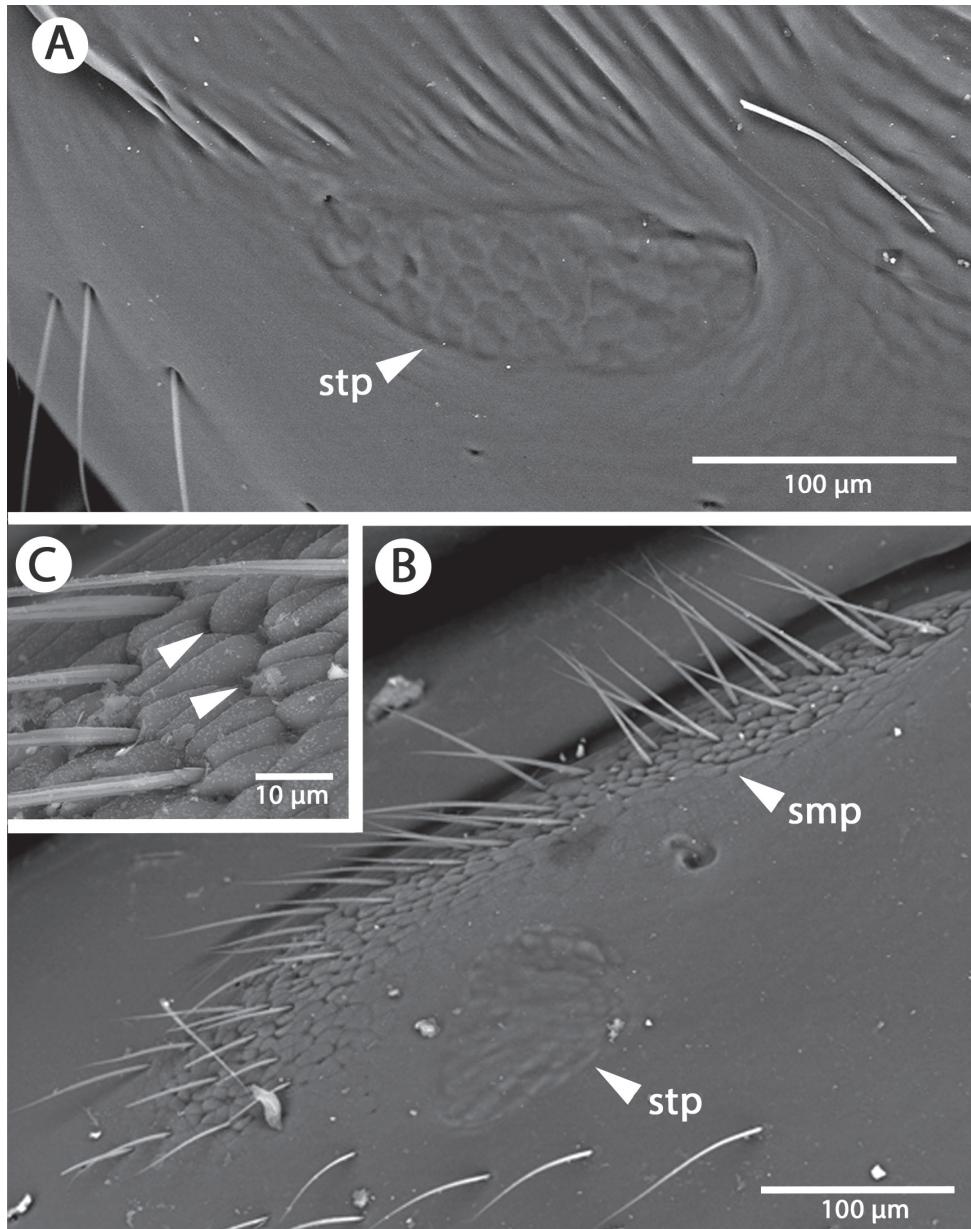
### Specimen observations, brightfield imaging and SEM Imaging

Translucent and setiferous patches were found in all Ceraphronoidea observed, including both males and females. SEM revealed that the syntergal and synsternal translucent patches lack setae and bear the impression of units that resemble epidermal cells, also known as scutes (Mikó et al. 2016) (Fig. 2A, B). No gland openings were found in the translucent cuticle. The syntergal and synsternal translucent patches occur in pairs on the metasoma. The translucent patches in each pair have roughly the same size and shape in all of the studied specimens, and appear to be bilaterally symmetrical. However, this is not the case for the syntergal and synsternal translucent patches, which additionally differ in size and shape between species (Fig. 1).

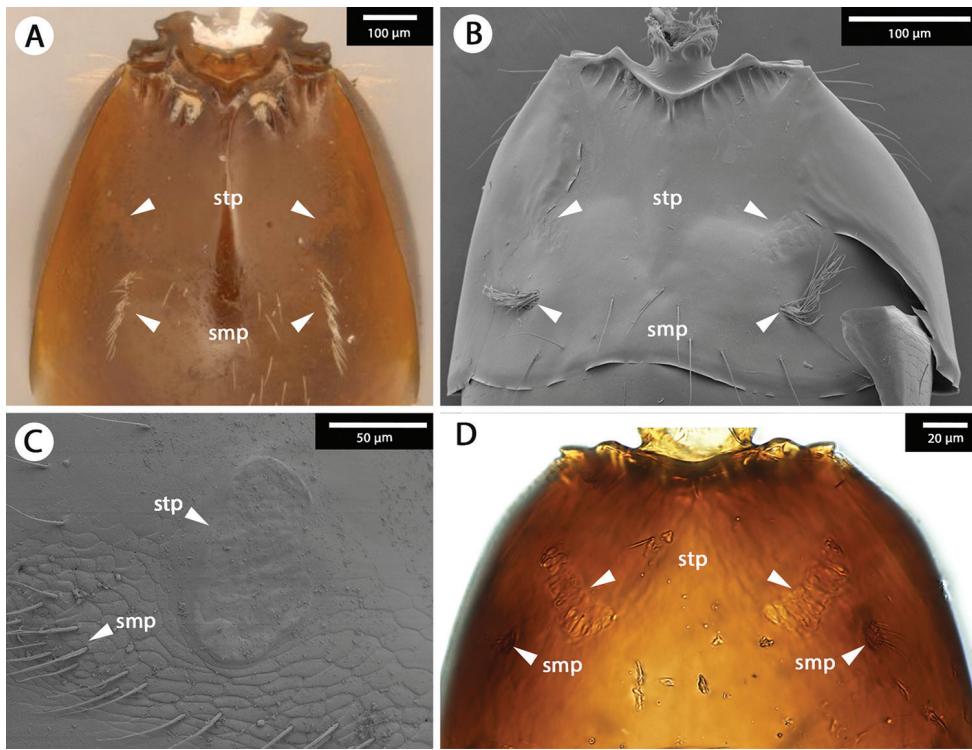
The synsternal setiferous patches also occur in pairs on the synsternite and are bilaterally symmetrical. Comparisons between different species of *Conostigmus* revealed species-specific differences in the length and shape of the setiferous patches (Fig. 1). SEM imaging also revealed the presence of scutes in the cuticle between the setae (Fig. 2B and C), as well as openings in the cuticle that could be related to gland function (Fig. 2C).

The location of the synsternal setiferous patches in relation to the synsternal translucent patches differs between the families and subfamilies of Ceraphronoidea (Figs 3–4; Mikó and Deans 2009; Mikó et al. 2013, 2016). In the family Ceraphronidae, the synsternal setiferous patches are located posterior to the synsternal translucent patches (Fig. 3A). This is also seen in the genus *Masner* (Fig. 3B). Within the family Megaspilidae, the locations of the synsternal patches differed between subfamilies (Figs 3–4; Mikó et al. 2013, 2016). In Megaspilinae, the synsternal setiferous patches occur laterally to the synsternal translucent patches (Fig. 3C), while in Lagynodinae, the synsternal setiferous patches are located anterior to the synsternal translucent patches (Fig. 4). The genus *Trassedia* has the synsternal setiferous patches located posterior to the synsternal translucent patches, similar to Ceraphronidae (Fig. 3D).

Observations of Orussidae revealed smooth patches of cuticle occurring on the anterior portion of both the second abdominal sternite and tergite. The patches were translucent in some specimens observed (Fig. 5A, B), while in others the patches were



**Figure 2.** SEM images of the syntergal and synsternal translucent patches and synsternal setiferous patches in male *Megaspilus armatus* Say, 1836 (Hymenoptera: Megaspilidae) specimens. **A** Dorsal surface of the metasoma, showing the scutes (identifier PSUC\_FEM 68527) **B** Ventral surface of the metasoma (identifier: PSUC\_FEM 50127) **C** Closer view of the synsternal setiferous patch and scutes, with arrows pointing to pore openings in the cuticle (identifier: PSUC\_FEM 50127) Abbreviations: smp = synsternal setiferous patch; stp = synsternal translucent patch.



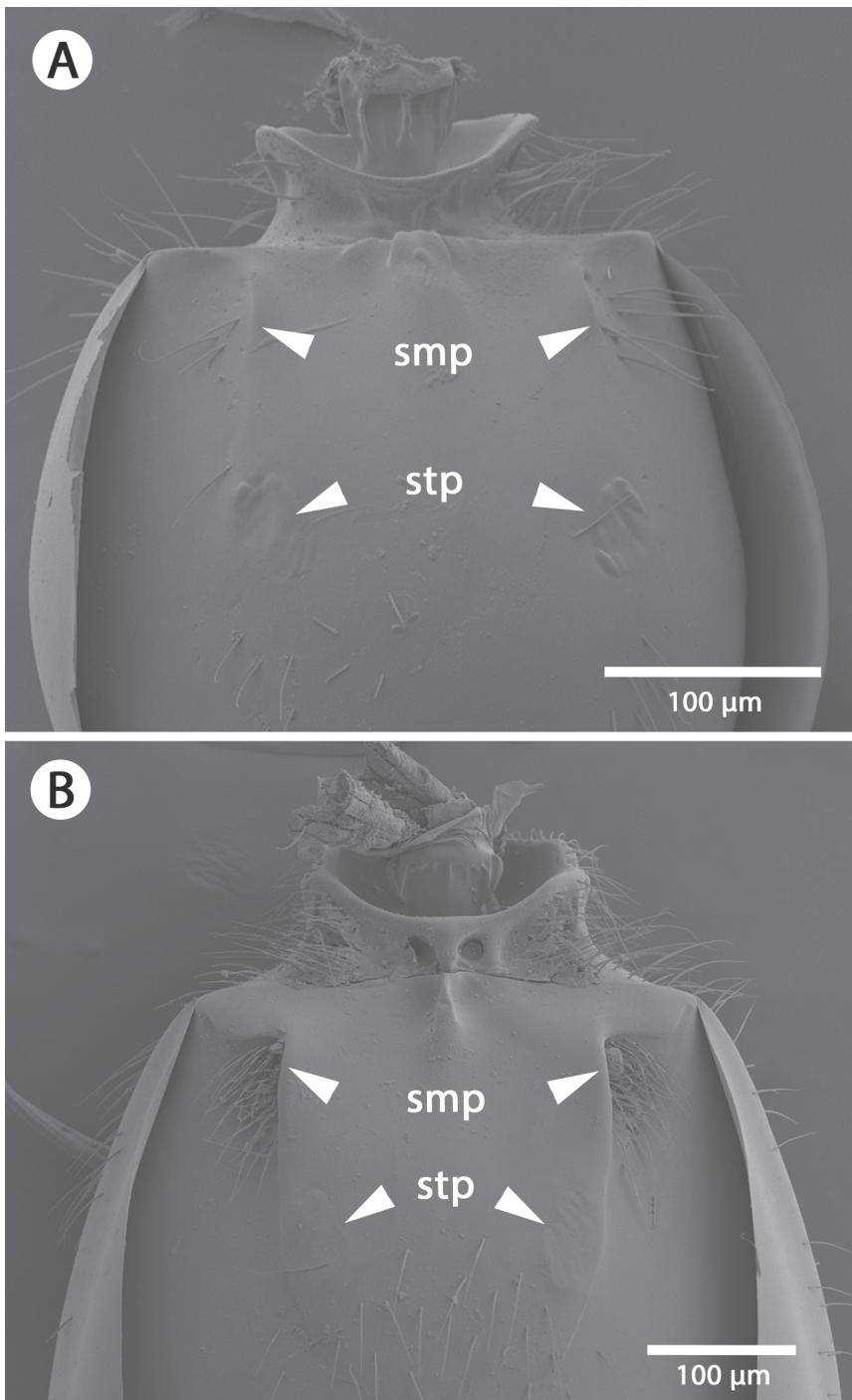
**Figure 3.** Images of the synsternal translucent and setiferous patches in the metasoma of different Ceraphronoidea. **A** Brightfield image of a *Ceraphron* sp. (Hymenoptera: Ceraphronidae) (identifier: PSUC\_FEM 27234) **B** SEM image of *Masner lubomirius* Deans & Mikó, 2015 (Hymenoptera: Ceraphronidae) (identifier: PSUC\_FEM 470955) **C** SEM image of *Trichosteres glabra* Boheman, 1832 (Hymenoptera: Megaspilidae) (identifier: IM 1512) **D** Brightfield image of a *Trassedia* sp. (Hymenoptera: Ceraphronidae) (identifier: IM 1109/ NCSU 71196) Abbreviations: smp = synsternal setiferous patch; stp = synsternal translucent patch.

the same color as the surrounding cuticle, and differed only in their surface sculpturing (Fig. 5C, D). The ventral patches of translucent cuticle were obscured by the hind coxa and only visible through dissection of the specimen (Fig. 5B). No patches of setae were observed near the patches of smooth or translucent cuticle on the tergite or sternite.

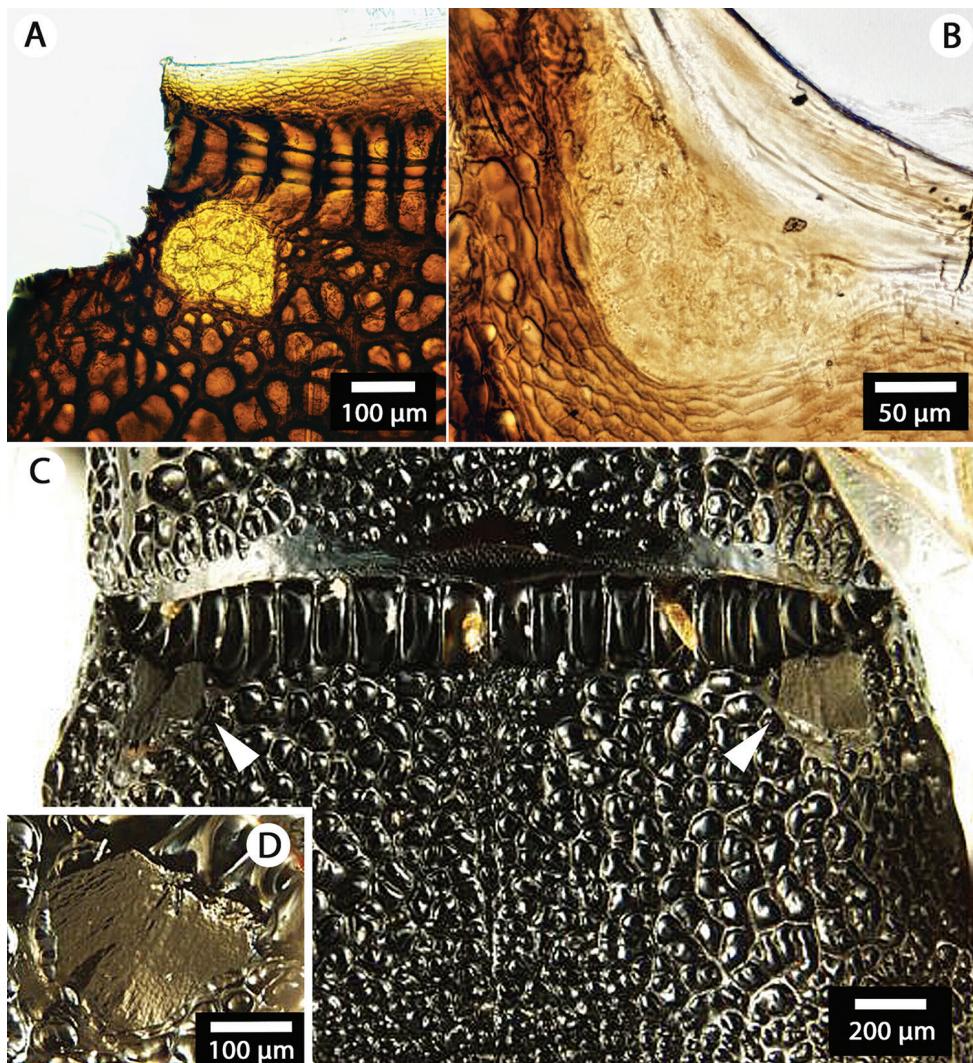
Patches of translucent cuticle were also observed in Ichneumonidae on the second metasomal tergite and sternite (Fig. 6). On the tergite, the appearance of these structures varied from patches to deep depressions. As in Orussidae, no patches of setae were found to be associated with the patches of translucent cuticle present on the tergite or sternite.

### Confocal Laser Scanning Microscopy (CLSM)

CLSM was used to check for the presence of resilin in the synsternal and syntergal translucent patches in male (Fig. 7A; animated version available on figshare at

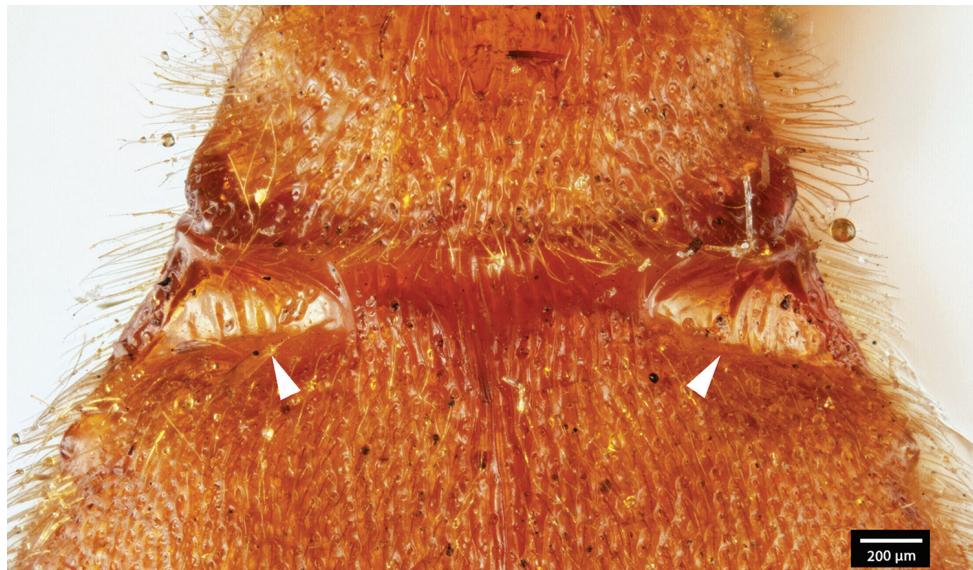


**Figure 4.** SEM image of the synsternal translucent patch and synsternal setiferous patch in a male (**A**) and female (**B**) *Lagynodes* sp. (Hymenoptera: Megaspilidae) Specimens from lot IM 930. Abbreviations: smp = synsternal setiferous patch; stp = synsternal translucent patch.

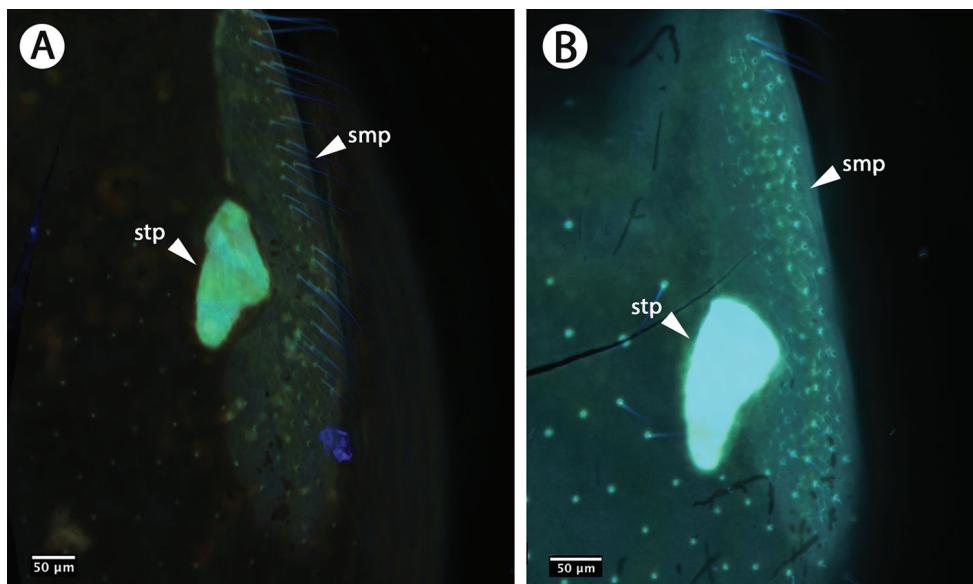


**Figure 5.** Brightfield images showing the dorsal and ventral patches of translucent cuticle in Orussidae, viewed externally. **A** Dorsal view of an *Orussus* sp. (Hymenoptera: Orussidae), viewed externally (identifier: IM 1445/ NCSU 53625) **B** Ventral view of the same specimen **C** Arrows pointing to dorsal patches of translucent cuticle in *Orussus abietinus* Scopoli, 1763 (Hymenoptera: Orussidae) (identifier: PSUC\_FEM 86200) **D** A closer view of one of the translucent patches from the same specimen.

<https://doi.org/10.6084/m9.figshare.4993820>) and female (Fig. 7B; animated version available on figshare at <https://doi.org/10.6084/m9.figshare.4993826.v1>) *Megaspilus armatus* Say, 1836 specimens. Though resilin was not detected, the translucent patches of cuticle fluoresced differently than the surrounding cuticle in



**Figure 6.** Brightfield images with arrows pointing to the patches of translucent cuticle in a *Trogus* sp. (Hymenoptera: Ichneumonidae) (identifier: PSUC\_FEM 86178).



**Figure 7.** CLSM image of the synsternal translucent patch and setiferous patch in a male (**A** identifier: PSUC\_FEM 86236) and female (**B** identifier PSUC\_FEM 86240) *Megaspilus armatus* Say, 1836 specimen (Hymenoptera: Megaspilidae), viewed externally. Abbreviations: smp = synsternal setiferous patch; stp = synsternal translucent patch.

both male and female specimens (Fig. 7). CLSM also revealed fluorescence of tissue underneath the cuticle of the setiferous patch that occurred in the same shape as the patch. The setae of the synsternal setiferous patch appeared to be rich in resilin, a feature that is shared among other setae present on the sternite. No differences were found between male and female specimens.

## Histology

Histological cross sections of the metasoma of *Dendrocerus* sp. and *Conostigmus* sp. (Hymenoptera: Megaspilidae) specimens revealed the presence of pore canals directly underneath the setae of the synsternal setiferous patches (Fig. 9). SBFSEM was used to build a three-dimensional model tracing a duct from a gland cell to an opening in the cuticle (Fig. 8A; animated version available at <https://doi.org/10.6084/m9.figshare.4004157.v1>). Closer inspection of the cuticle with TEM revealed smaller pores fringed with cells containing smooth endoplasmic reticulum (Fig. 9).

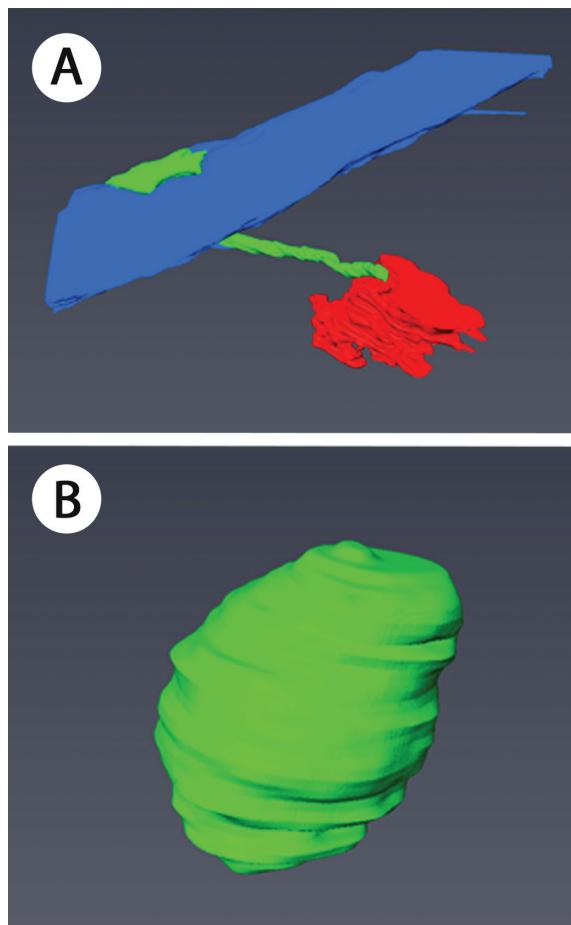
The internal structures associated with the synsternal translucent patches were different than those of the synsternal setiferous patches. Histological cross sections did not show any pore canals in the translucent cuticle. However, TEM and SBFSEM revealed membrane-bound structures with excess membrane folds present underneath the translucent patches (Fig. 10). SBFSEM was used to build a three dimensional model of one of these structures, revealing it to have a rounded shape (Fig. 8B; animation available at <https://doi.org/10.6084/m9.figshare.4993832.v1>).

## Discussion

### Overview of the setiferous patches

Histological cross sections of the metasoma of *Dendrocerus* sp. and *Conostigmus* sp. (Hymenoptera: Megaspilidae) specimens revealed pore canals directly underneath the setae of the synsternal setiferous patches (Fig. 9). These appear to be class 3 gland cells, each consisting of a gland cell and a secretory duct that connects it to the cuticle (Noirot and Quennedey 1974; Quennedey 1998). Using SBFSEM, a three-dimensional model was built tracing a duct from a gland cell to an opening in the cuticle (Fig. 8A; animated version available at <https://doi.org/10.6084/m9.figshare.4004157.v1>). This confirms the presence of gland cells underneath the synsternal setiferous patches within Ceraphronoidea.

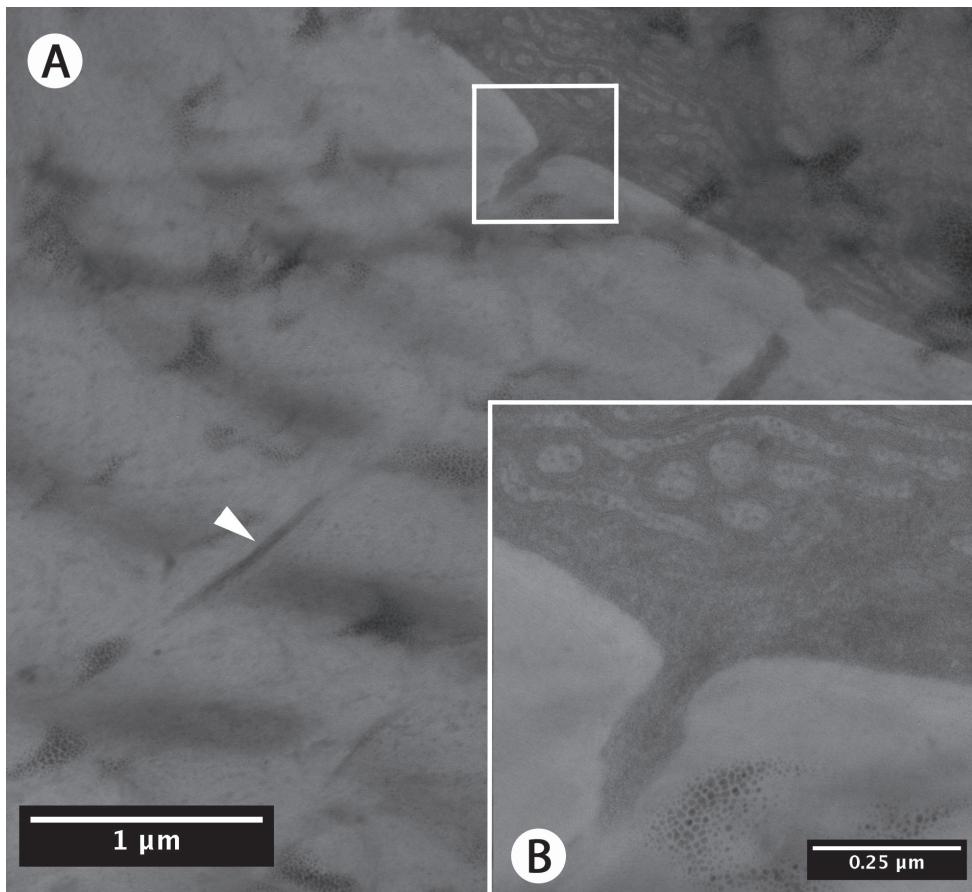
Closer inspection of the cuticle with TEM revealed smaller pores fringed with cells containing smooth endoplasmic reticulum (Fig. 9B), often found in gland cells producing pheromones or lipids (Quennedey 1998). These appear to be class one gland cells, which adjoin the cuticle and secrete products either directly through the cuticle



**Figure 8.** **A** A three-dimensional model of a class 3 gland cell found underneath the cuticle. The model shows the cuticle in blue, the gland cell in red, and then secretory duct connecting them in green **B** A three-dimensional model of a lamellar body.

or through epicuticular canals (Noirot and Quennedey 1974; Quennedey 1998). Since both class one and class three gland cells are present, the gland underneath the synsternal setiferous patch appears to be a composite gland comprised of multiple gland cell types (Noirot and Quennedey 1974). CLSM imaging may even show the outline of this gland, fluorescing underneath the cuticle in the same shape as the synsternal setiferous patch (Fig. 7A). Patches of setae have long been known to be associated with glandular activity in Hymenoptera (Buckingham and Sharkey 1988; Debolt 1973; Mikó et al. 2010; Noirot and Quennedey 1974), but this is the first time that this has been confirmed in Ceraphronoidea.

Glands underneath patches of setae in Hymenoptera are thought to secrete pheromones, with the setae acting to increase the surface area for diffusion of these secretions



**Figure 9.** **A** TEM image of the class one gland cells found underneath the synsternal translucent patch in a *Dendrocerus* sp. (Hymenoptera: Megaspilidae). The arrow points to the secretory duct in the cuticle, while the square outlines the gland cells at the base of these ducts **B** A closer look at the class one gland cells at the base of one of these ducts. Specimen identifier: IM 5442.

(Debolt 1973; Mikó et al. 2007; Noirot and Quennedey 1974). Pheromones have a wide variety of important ecological, behavioral, and physiological functions in insects (Howard and Blomquist 2005). One possible function of these glands could be the production of defensive pheromones, which has been proposed for abdominal glands in Ichneumonoidea (Buckingham and Sharkey 1988; Townes 1939). It is also possible that these abdominal glands could produce pheromones that play roles in courtship, mate recognition or sexual selection, which has been found in other parasitoid wasps (Nieuwkoop et al. 2013; Rutherford et al. 2007, 2009). There may be behaviors associated with these patches as well. Very little is known about the behavior of Ceraphronoidea, but the pumping motion of the abdomen in Braconidae has been proposed to be associated with abdominal glands (Buckingham 1968; Buckingham and Sharkey 1988) and could help to disperse pheromones.

## Overview of translucent cuticle in Ceraphronoidea

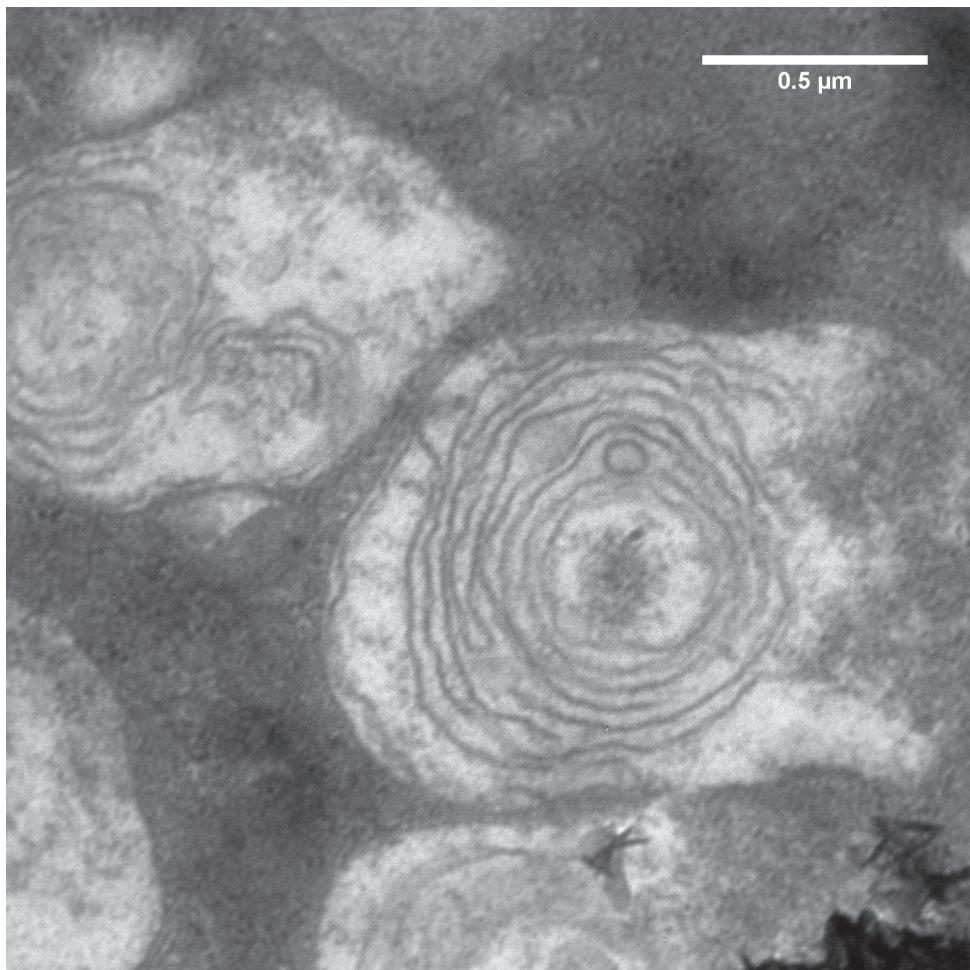
Translucent cuticle over the compound eyes and ocelli in insects often contains resilin, a structural protein that autofluoresces between 320 nm and 415 nm (Andersen 1963; Michels and Gorb 2012), allowing for detection and visualization using CLSM (Deans et al. 2012). CLSM of the translucent patches did not indicate resilin; however, the patches fluoresced differently than the surrounding cuticle, indicating that the patch has a different structural composition. Though the patches do not appear to contain resilin, they may contain a resilin-derivative or other protein involved in the structure of translucent and transparent cuticle, such as crystallin (Janssens and Gehring 1999). The identity of what makes the patch fluoresce differently remains to be determined.

The internal structures associated with the synsternal translucent patches were different than those of the synsternal setiferous patches. Histological cross sections did not show any pore canals in the translucent cuticle. However, TEM and SB-FSEM revealed membrane-bound structures with excess membrane folds present underneath the translucent patches (Fig. 10). These structures were identified as lamellar bodies, which are membrane-bound structures with excess membrane folds that are produced when fat bodies or vacuoles are broken down (McDermid and Locke 1983; Quennedey 1998; Vigneron et al. 2014). A three-dimensional model of one of these lamellar bodies was built using SBFSEM, revealing that these structures have a rounded shape (Fig. 8B; animation available at <https://doi.org/10.6084/m9.figshare.4993832.v1>).

Lamellar bodies are involved in organelle recycling, and can have glandular functions such as storage and secretion (McDermid and Locke 1983; Quennedey 1998; Vigneron et al. 2014). Lamellar bodies can also be associated with photoreceptors (White 1968). Extraocular photoreceptors, which are photoreceptors found outside of the eyes and ocelli, have been found underneath translucent cuticle in other insects (Williams and Adkisson 1964). It is possible that there may be extraocular photoreceptors underneath these patches of cuticle, and that the cuticle is transparent to allow light into the metasoma. Such a system could be important for the regulation of circadian rhythms or for sensing seasonal changes in photoperiod (Mizoguchi and Ishizaki 1982; Renninger et al. 1997). This system has never been described before in Ceraphronoidea; any further work investigating the translucent cuticle offers a high potential for new discovery.

## Phylogenetic relevance of the syntergal and synsternal translucent patches

Both patches of setae (Masner and Huggert 1989; Mikó et al. 2010) and patches of translucent cuticle (Liu et al. 2006) have been used to distinguish between species and even genera in other Hymenoptera (Vilhelmsen 2003). The same is true within Ceraphronoidea. Work done on the genera *Conostigmus* and *Dendrocerus* (Hymenoptera: Megaspilidae) revealed differences in the shape and size of translucent and setiferous patches of cuticle between species (Dessart 1997, 1999, 2001), making differences in



**Figure 10.** TEM image of the lamellar bodies found underneath the synsternal translucent patch in a *Dendrocerus* sp. (Hymenoptera: Megaspilidae) Specimen identifier: IM 5442.

their structure a potent diagnostic feature to distinguish between species. To date, there are no indications of sexual dimorphism concerning these characters, making it possible to match males and females in situations where only one sex has been described.

There are also differences in the locations of the setiferous and translucent patches between different members of Ceraphronoidea. The synsternal setiferous patches are located posteriorly to the synsternal translucent patches in the family Ceraphronidae, laterally to them in the Megaspilinae, and anteriorly to them in Lagynodinae. It is unclear why these structures occur in different locations across different groups. If the setiferous patches secrete substances that play a role in courtship or defense, the locations of the setiferous patches could indicate different courtship or defensive behaviors in different groups. It is also possible that these structures could have evolved

independently. The phylogenetic relationships between the families and subfamilies in Ceraphronoidea are unresolved (Mikó et al. 2013). The variation in the locations of the translucent and setiferous patches are consistent in each subfamily and could represent a putative synapomorphy for each lineage, thought further analysis is needed to verify the phylogenetic relevance of these structures.

The locations of the translucent and setiferous patches in relation to each other could also provide relevant information concerning the placement of difficult genera within Ceraphronoidea. The genus *Masner* is perplexing in that it shares characters with both Megaspilidae and Ceraphronidae. It is thought to be sister to Ceraphronidae (Mikó and Deans 2009; Mikó et al. 2013), a hypothesis which is supported by our findings in the relative location of the synsternal setiferous and translucent patches. Another perplexing genus with uncertain placement within Ceraphronoidea is *Trassedia*, which was formerly grouped with Megaspilidae based on the presence of a pterostigma and nine flagellomeres in females (Cancemi 1996). However, based on other morphological characters, such as the presence of Waterston's evaporatorium, a single mesotibial spur, and axillular setae, and absence of an occipital depression and a narrow sclerite anterior to the synsternum, the genus is now thought to be part of Ceraphronidae (Mikó et al. 2013). In *Trassedia*, the synsternal setiferous patches occur posteriorly to the synsternal translucent patches as in Ceraphronidae supporting placement in that family (Mikó et al. 2013).

Whereas patches of setae have long been known to be associated with glandular openings in Hymenoptera (Buckingham and Sharkey 1988; Debolt 1973; Mikó et al. 2010; Noirot and Quennedey 1974), and are found in several distantly related groups ranging from Megachilidae (Noirot and Quennedey 1974) and Mutillidae (Debolt 1973) to Platygastroidea (Mikó et al. 2010), the presence of translucent patches of cuticle in the metasoma is much less common. As the putative sister to Apocrita, Orussidae represent an important step in the evolution of Hymenoptera and the parasitoid lifestyle (Mao et al. 2014; Peters et al. 2017; Sharkey 2007). Orussidae also have patches of smooth cuticle similar to the syntergal and synsternal translucent patches found in Ceraphronoidea. Just as in Ceraphronoidea, the patches in Orussidae occur in pairs and are found both dorsally and ventrally. Their location in Orussidae (second abdominal tergite and sternite) is comparable to their location in Ceraphronoidea (first metasomal tergite and second metasomal sternite). Based on these similarities, the structures in Orussidae and Ceraphronoidea may have similar functions or evolutionary origins.

Similar structures are also present in Ichneumonidae on both the dorsal and ventral surfaces of the metasoma. The structures present on the tergite are known by different names. According to Townes (1969), these structures are known collectively as the thyridia, described as patches of cuticle with different surface sculpturing occurring in pairs anterior to the spiracle on the second tergite. However, Goulet and Huber (1993) identifies these structures as the gastrocoeli, and considers the thyridia to be the unique cuticular structure specific to the gastrocoeli. Whether these translucent patches are called the gastrocoeli or the thyridia, both authors note that they differ between ich-

neumonoids and are not always present (Goulet and Huber 1993; Townes 1969). In Ichneumonidae, the thyridia or gastrocoeli are located on the second tergite, not on the first as in Ceraphronoidea or Orussidae. However, in Ceraphronoidea, the first tergite is longer than the successive tergites, and may have been formed by the fusion of multiple tergites; thus, the structures may be comparable between Ceraphronoidea and Ichneumonidae, and may also have similar functions or evolutionary origins.

## Acknowledgments

The authors would like to thank Missy Hazen for her expertise and assistance with CLSM, TEM and SBFSEM at the Penn State Microscopy and Cytometry Facility (University Park, PA), John Catolina for his expertise and assistance with SEM at the Penn State Microscopy and Cytometry Facility (University Park, PA), and Julie Anderson for her expertise and assistance with SEM at the Penn State Materials Research Institute (University Park, PA). This work was also performed in part at the Analytical Instrumentation Facility (AIF) at North Carolina State University, which is supported by the State of North Carolina and the National Science Foundation (award number ECCS-1542015). The AIF is a member of the North Carolina Research Triangle Nanotechnology Network (RTNN), a site in the National Nanotechnology Coordinated Infrastructure (NNCI). The authors would like to thank Lars Vilhelmsen for his expertise on Orussidae, Emily Sandall for her assistance with GBIF and Michael J. Sharkey for his gift of specimens. Special thanks to the Frost Entomological Museum (PSUC), the North Carolina State University Insect Museum (NCSU), the Wisconsin Insect Research Collection (WIRC), the American Museum of Natural History (AMNH), and the C.A. Triplehorn Insect Collection at the Ohio State University (OSUC) for the loans of specimens. The authors would also like to thank Lars Vilhelmsen, Christian Wirkner, Lars Krogmann, and Matthew Yoder for their valuable input in improving the manuscript. This material is based upon work supported by the U. S. National Science Foundation, under Grant Numbers DBI-1356381 and DEB-1353252. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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## Supplementary material I

### Specimen locality information

Authors: Carolyn Trietsch, István Mikó, Jonah M. Ulmer, Andrew R. Deans

Data type: specimens data

Explanation note: A table listing all of the specimens used in this study, and their associated locality and repository information.

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Link: <https://doi.org/10.3897/jhr.60.13692.suppl1>