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Source: American Museum Novitates, 2019(3920) : 1-28

Published By: American Museum of Natural History

URL: <https://doi.org/10.1206/3920.1>

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Unique Metasomal Musculature in Sweat Bees (Hymenoptera: Apoidea: Halictidae) Revealed by Micro-CT Scanning

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ABSTRACT

Bees of the family Halictidae (Apoidea: Anthophila) have three pairs of thick, bundled muscles that are circular to subcircular in cross section within the first metasomal segment, as revealed by micro-CT scanning of 16 species in 15 genera of five bee families. In nonhalictids and the basal halictid subfamily Rophitinae, these muscles are planar (flat and sheetlike), typically lying between the anterior air sacs and abdominal wall. In Nomiinae and Halictinae, these muscles, especially the dorsal-ventral pair, bulge into air-sac space, partly enveloped by air-sac membrane. A possible function may be to facilitate metasomal compression and contraction, and thus air flow. The bundled shape of these derived halictid muscles is similar to that of flight muscles, but further data is needed to determine if they are fibrillar, which would suggest a completely different function.

INTRODUCTION

For centuries, external (cuticular) morphology has been the primary tool for insect systematics. In the seminal volumes of the arthropod anatomist Robert E. Snodgrass (1875–1962) (e.g., Snodgrass, 1935, 1956) dissection was the method of choice and by necessity to examine

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internal insect anatomy, and these have remained the primary works for generations. In the past several years, however, microcomputed tomography, or μ CT scanning, has enabled new insights into previously unexplored areas of both living and fossil arthropod anatomy (e.g., Friedrich and Beutel, 2008; Greco et al., 2008, 2011; Garwood and Dunlop, 2014).

Micro-CT of insects is especially useful because of its high resolution, nondestructive nature, and the complexity and small size of some hexapod organ systems. Examination of internal anatomy via dissection requires the cutting and movement of cuticular structures, likely distorting fine, membranous elements such as air sacs and tracheae. Thin-sectioning combined with microscopy can yield impressive detail but is tedious and time consuming. The skill required in both the sectioning and photography of sections for later registration and tomographic processing makes this technique generally inaccessible. Micro-CT, however, rapidly captures fine-grained detail of structures and tissue density differences in situ. Identifying morphology of air spaces, for example, is facilitated by the fact that the air space density is significantly lower than the surrounding musculature and integument of the insect specimen, allowing in situ investigations that would be difficult or impossible using conventional means.

Many families of flying insects possess abdominal air sacs, presumably used for both weight relief and respiration. Bees are certainly no exception, as seen in species of the genus *Lasioglossum* (*Dialictus*) (fig. 1). In this study, an examination of abdominal air-sac anatomy in bees using μ CT revealed some interesting features that appear to be restricted to a clade within Halictidae.

Halictidae are one of the most diverse families among bees, with a range of biologies spanning from specialized oligoleges, cleptoparasites, and nocturnal and crepuscular species to those that live in eusocial societies (Michener, 2007). Abundant in habitats ranging from deserts to rain forests, they can be found on every continent except Antarctica, and are commonly referred to as “sweat bees” owing to the occasional predilection of some to lap perspiration on the skin. Halictidae also contain one of the largest of animal genera, *Lasioglossum* Curtis s.l. (Halictinae: Halictini), with over 1800 species worldwide (Michener, 2007). Although relationships between subfamilies and tribes in Halictidae continue to be actively studied, the family has been agreed upon as monophyletic for decades (Michener, 2007). Of the three monophyletic subfamilies, Ropitinae, Nomiinae, and Halictinae, Rophitinae are the most basal. Halictinae, an important subject of studies regarding eusociality (Michener, 1974; Gibbs et al., 2012; Schwarz et al., 2007) are further subdivided into the tribes Augochlorini, Caenohalictini, and Halictini, with the last sometimes further divided into subtribes (or less often individual tribes) Halictina, Gastrohalictina, Sphecodina, and Thrinchostomatina (Pesenko, 1999; Engel, 2001, 2005; Michener, 2007).

In a comparison of the metasomal anatomy of various bees, unusually large muscles were observed in the first segment of the metasoma of some specimens. Rather than the thin, planar muscle sheets usually associated with abdominal contraction, these are cylindrical in nature, comprised of bundles of fibers and enveloped by the membranes of the metasomal air sacs in a manner similar to thoracic flight muscles (fig. 2). Subsequent scans indicated that the size, placement, and structure of the muscles appeared to be restricted to a derived subset of Halictidae, as examination of other bee families did not show this cylindrical, bundlelike structure.

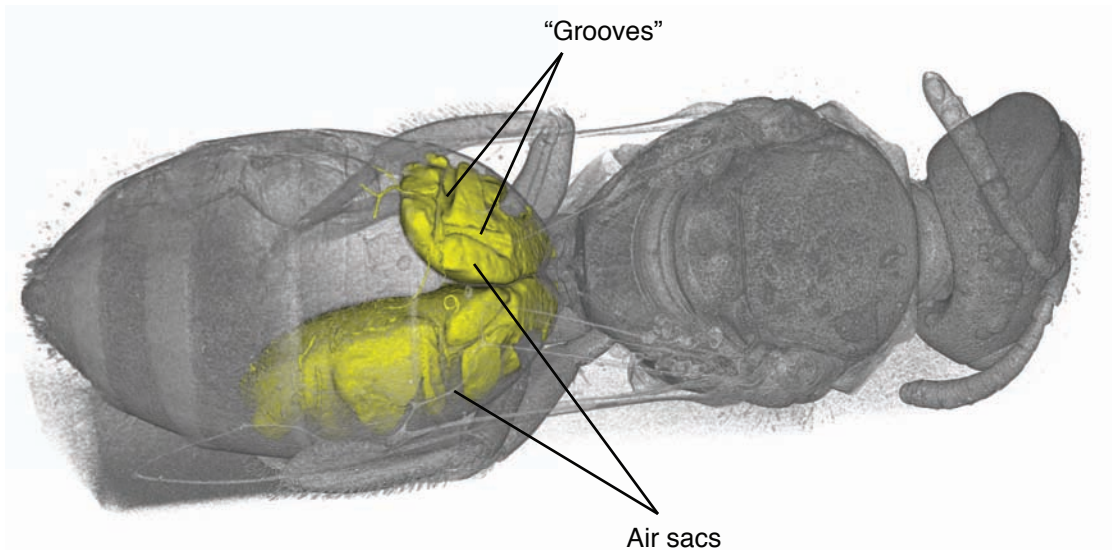


FIGURE 1. Volume rendering of *Lasioglossum (Dialictus)* sp. (Halictinae: Halictini), showing location of abdominal air sacs, displayed as a yellow solid, and “grooves” where metasomal muscles extend into the air-sac space. The insect’s exoskeleton and internal anatomy is rendered translucent, allowing examination of air-sac morphology. The bilateral asymmetry shown here is not uncommon, and usually has to do with how recently the specimen has fed. Distension of the gut can occupy space normally taken up by air sacs.

Herein we describe the location and structure of these muscle groups, how these groups appear to be different from other bee families, and suggest future work to discern their composition and function.

MATERIAL AND METHODS

MICRO-CT SCANNING AND ANALYSIS

Specimens from the collection of the Division of Invertebrate Zoology, American Museum of Natural History, New York (AMNH), were scanned on site at the AMNH Microscopy and Imaging Facility. Scans were taken with a GE Phoenix v|tome|x s240 (Germany), equipped with either a 240 or 180 kV X-ray source. A diamond or molybdenum target was used, depending on the specimen and scanning conditions. Scanning parameters for all specimens are detailed in table 1.

Dried pinned specimens from the AMNH collection and freshly collected frozen specimens obtained by the authors in Manhattan, New York City, were used in the study. Frozen specimens were thawed to room temperature prior to scanning, and pinned specimens were temporarily removed from the metal pin. Specimens were placed in plastic vials and stabilized with various packing materials to immobilize them as much as possible during the scan. Movement of specimens during scans is undesirable and can result in blurred images, requiring a repeat scan. After scanning, pinned specimens were repinned and replaced into the collection with voucher specimen labels to indicate that scans had been performed. Frozen specimens were returned to cold storage for later study.

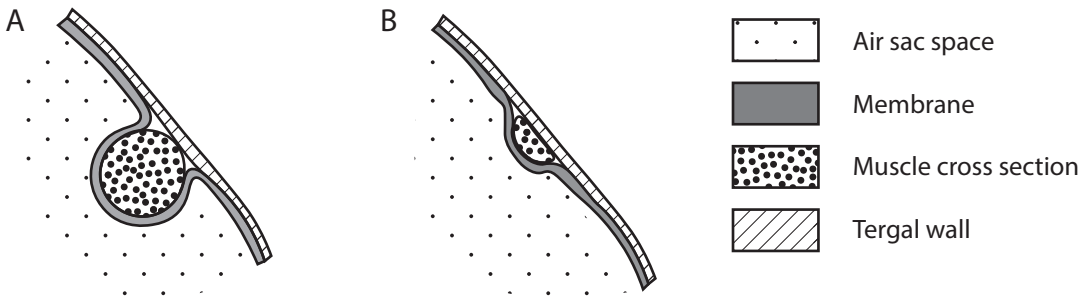


FIGURE 2. Representation of metasomal muscle cross section seen in: **A.** Halictidae, compared with **B.** typical arrangement.

For all specimens, volume reconstruction from raw projections was performed using `data|x 2.3.2`. A combination of manual and semiautomatic geometry correction was used for each specimen. Reconstructed volumes were exported as 16-bit tiff stacks for postprocessing. Specimens with a less than optimal orientation for visualization were rotated using FIJI (Schindelin et al., 2012) and exported in NRRD format prior to segmentation and rendering.

Segmentation and volume rendering was done using 3D Slicer 4.9 (Fedorov et al., 2012). Typically, the first step in the visualization process is “vial removal.” With a density similar to insect cuticle, removal of the vial and packing material used to hold and immobilize the specimen is necessary for visualization using volume rendering. Failure to remove these materials results in a high-resolution image of the vial. Masking and cropping of the dataset was achieved using a “fill between slices” technique, where the desired sample area was marked at representative slices spaced throughout the volume along the posterior-anterior lateral axis of the specimen. A “masking volume” was then created by interpolating across these slices using a morphological contour interpolation method (Zukić et al., 2016). When applied against the original volume, the “mask scalar volume” module removed the unwanted vial and packing material, leaving only the insect. At this point, standard volume-rendering techniques were used to view the specimen. Clipping planes allowed examination of detailed anatomy of internal structures. Figures were composed and annotated with Adobe Photoshop CC 2018, and cladograms were prepared using Adobe Illustrator CC 2018.

“Cutaway” volume-rendered views for pinned specimens were used to determine muscular structure in the metasoma. Segmentation techniques were required for frozen specimens as gut contents obscured direct viewing of muscle morphology. The method used for cropping and volume-rendering for presentation of internal structure in successive figures is shown in figure 3. An example of segmentation for frozen specimens is shown in figure 4.

TERMINOLOGY

Following the convention of Michener (2007), the first metasomal segment is the segment behind the propodeal-metasomal constriction, and is referred to as T1. Successive posterior segments are designated T2, T3, and so on. “Origin” and “insertion” points for insect muscles can be somewhat nonspecific, especially for muscles that do not span segments (metameres)

TABLE 1. Scanning parameters for all specimens. All selected taxa are female with the exception of those labeled ($\delta/\text{♀}$), where both male and female specimens were scanned.

Taxon	Family	Subfamily	Tribe	Voltage	Current	Aver-aging	Skip	Exposure	Voxel size	Preser-vation	AMNH Bee Barcode
<i>Megalopta nitidicollis</i>	Halictidae	Halictinae	Augochlorini	100 kV	200 μA	4	1	333 mSec	9.0 μm	Pinned	AMNH_IZC00290957
<i>Augochlorella aurata</i>	Halictidae	Halictinae	Augochlorini	100 kV	200 μA	4	1	400 mSec	4.8 μm	Pinned	AMNH_BEE00111455
<i>Augochlora pura</i>	Halictidae	Halictinae	Augochlorini	70 kV	210 μA	4	1	400 mSec	9.5 μm	Frozen	
<i>Lasioslossum</i> sp.	Halictidae	Halictinae	Halictini	70 kV	220 μA	3	0	400 mSec	5.4 μm	Frozen	
<i>Thrinchosoma torridum</i>	Halictidae	Halictinae	Halictini	100 kV	200 μA	4	1	400 mSec	9.1 μm	Pinned	AMNH_IZC00290958
<i>Pseudagapostemon citricornis</i> (♀)	Halictidae	Halictinae	Halictini	100 kV	200 μA	4	1	400 mSec	6.1 μm	Pinned	AMNH_IZC00110061
<i>Pseudagapostemon citricornis</i> (δ)	Halictidae	Halictinae	Halictini	70 kV	285 μA	3	1	400 mSec	4.8 μm	Pinned	AMNH_BEE00042071
<i>Sphecodes albilabris</i> (♀)	Halictidae	Halictinae	Halictini	90 Kv	220 μA	4	1	400 mSec	7.2 μm	Pinned	AMNH_IZC00290964
<i>Sphecodes albilabris</i> (δ)	Halictidae	Halictinae	Halictini	90 Kv	220 μA	4	1	400 mSec	6.5 μm	Pinned	AMNH_IZC00290963
<i>Caenohalictus rostraticeps</i>	Halictidae	Halictinae	Caenohalictini	100 kV	200 μA	4	1	400 mSec	5.7 μm	Pinned	AMNH_IZC00290962
<i>Dieunomia kirbii</i> (♀)	Halictidae	Nomiinae	Dieunomiini	100 kV	200 μA	4	1	400 mSec	14.4 μm	Pinned	AMNH_IZC00290959
<i>Dieunomia kirbii</i> (δ)	Halictidae	Nomiinae	Dieunomiini	70 kV	285 μA	4	1	400 mSec	8.2 μm	Pinned	AMNH_IZC00290757
<i>Dieunomia arizonensis</i>	Halictidae	Nomiinae	Dieunomiini	100 kV	200 μA	4	1	400 mSec	10.2 μm	Pinned	AMNH_BEE00046643
<i>Xeralictus bicuspidariae</i> (♀)	Halictidae	Rophi- tinae	Rophitini	100 kV	200 μA	4	1	400 mSec	5.8 μm	Pinned	AMNH_IZC00177862
<i>Xeralictus bicuspidariae</i> (δ)	Halictidae	Rophi- tinae	Rophitini	70 kV	285 μA	4	1	400 mSec	4.9 μm	Pinned	AMNH_IZC00290758
<i>Osmia bucephala</i>	Megach- ilidae	Mega- chilinae	Osmiini	70 kV	220 μA	3	0	400 mSec	12.4 μm	Pinned	AMNH_BEE00016321
<i>Protoxaea gloriosa</i>	Andreni- dae	Oxaeinae		70 kV	220 μA	3	0	400 mSec	14.0 μm	Pinned	AMNH_BEE00048808
<i>Centris americana</i>	Apidae	Apinae	Centridini	70 kV	220 μA	3	0	400 mSec	19.4 μm	Pinned	AMNH_IZC00290960
<i>Melipona grandis</i>	Apidae	Apinae	Meliponini	100kV	200 μA	4	1	400 mSec	10.8 μm	Pinned	AMNH_IZC00290961
<i>Colletes thoracicus</i>	Colletidae	Colletinae		70 kV	220 μA	3	0	400 mSec	13.5 μm	Pinned	

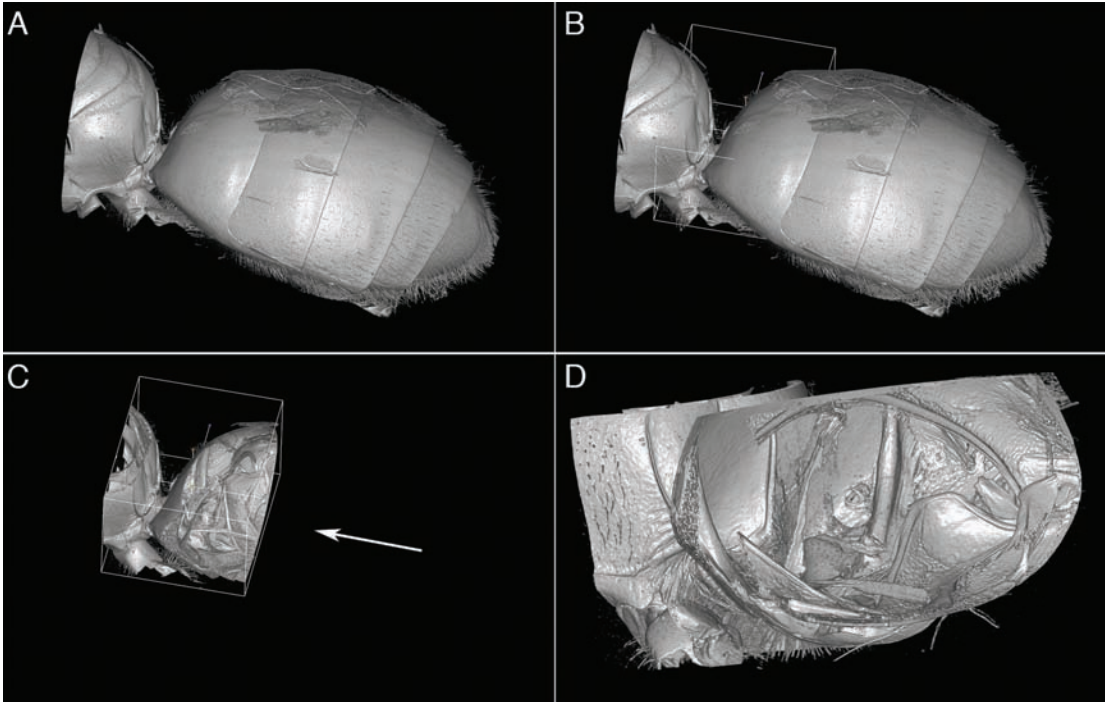


FIGURE 3. Volume renderings of *Megalopta* sp. (Halictinae: Augochlorini), detailing cropping method used to show metasomal muscle morphology. **A**, Full specimen. **B**, Full specimen with location of clipping box shown. The location is not identical for all specimens, but the box shown here is representative for specimen cutaways shown in following figures. **C**, Clipped specimen, shown in same location. Arrow denotes direction of view in **D**, toward posterior. **D**, Rotated and scaled posterior view, showing details of internal structures. Orientations and clipping locations that differ significantly from those shown are described in subsequent figure captions.

or operate appendages. For this discussion, the following conventions are used. Each muscle occurs in pairs:

- Dorsal-ventral muscles (*dvm*): origin is on the tergum inner wall, insertion is on sternum.
- Dorsal-longitudinal muscles (*dln*): origin is on the anterior inner wall of tergum, insertion is on antecosta of following tergum.
- Oblique muscles (*om*): origin is on anterior wall of tergum, insertion on antecosta of following sternum.
- Spiracles (*sp*) are designated in figures where they are visible.
- The inner wall of the tergum (*tw*) and antecosta (*ac*) are designated in some figures to serve as landmarks.

The abbreviations *dln*, *dvm*, and *om* are used throughout, including figure captions. In addition to various representatives of major halictid clades, five outgroup taxa were examined, all of which were pinned specimens from the AMNH collection. After scanning, specimens were labelled “VOUCHER SPECIMEN Micro-CT Scanned IV-V/2018 D. Grimaldi, S. Davis, & H. Herhold” and returned to the collection as vouchers.

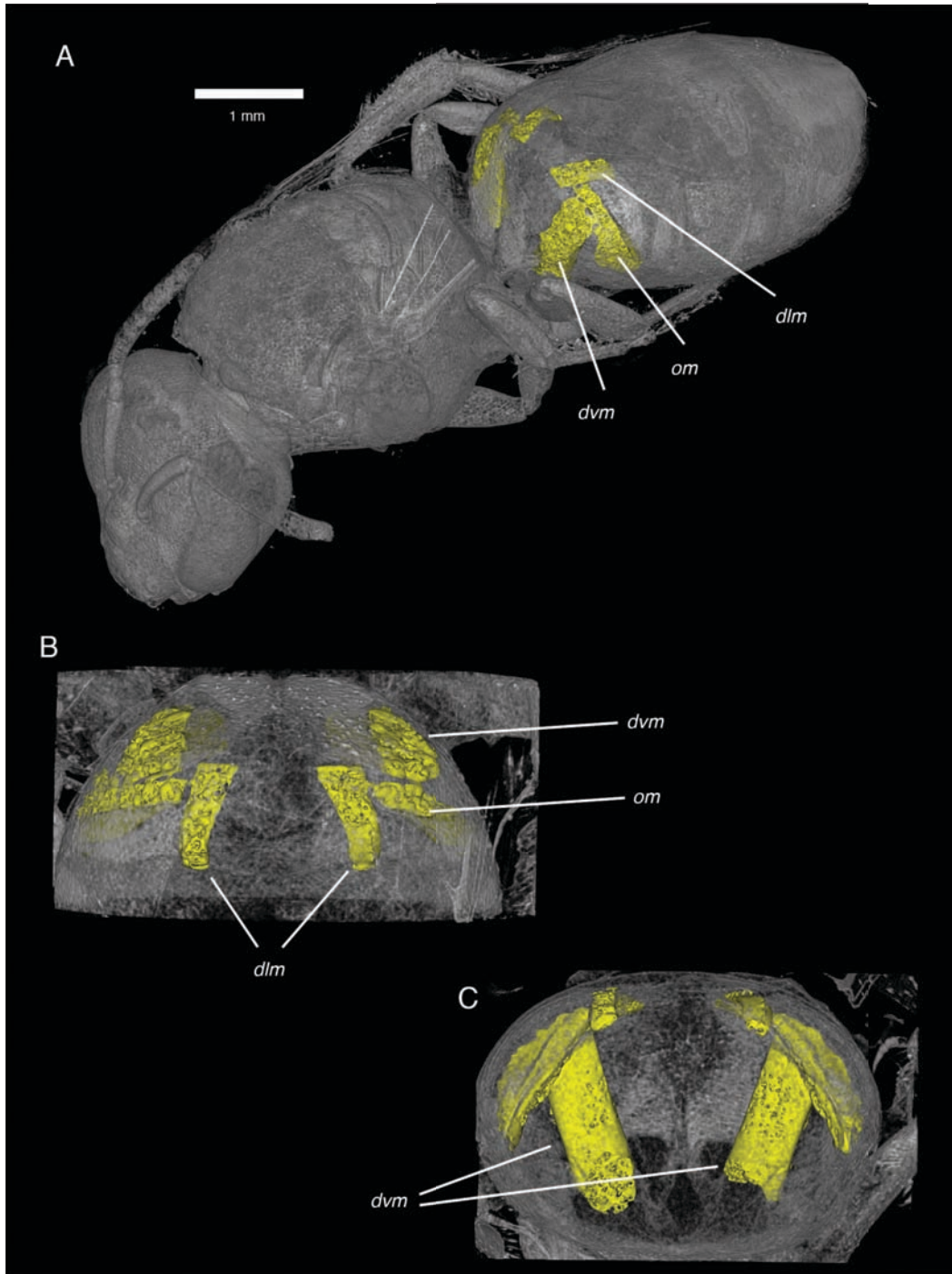


FIGURE 4. Transparent volume renderings of *Augochlora pura* (Say) (Halictinae: Augochlorini), detailing location of metasomal muscle groups. **A.** Full specimen. **B.** Dorsal close-up view of T1 muscle groups. **C.** Posterior view (looking from sting toward head) of T1 muscle groups.

SELECTION OF SPECIMENS

A total of 16 species were scanned for the study: 11 Halictidae (mostly females but also four males), two Apidae, and one each in Andrenidae, Megachilidae, and Colletidae. Taxa were chosen to represent subfamilial and tribal diversity within Halictidae, as well as to determine homologues in closely related families. Several specimens of “outgroup” species were selected to better ascertain possible muscle similarities among bee families. For some taxa males were also scanned to investigate the possibility of sexual dimorphism.

As mentioned previously, examination of both external and internal anatomy of arthropods using micro-CT is an extremely powerful technique. Of great importance to this study, muscle tissue resolves extremely well, providing detailed visualization of morphology. Dried specimens featured exceptional preservation of muscle tissue. Fine-scale detail of banding is readily apparent, enabling the location and identification of muscles and homology across taxa. Occasional preservation defects were visible, in particular, fracturing of dorsal-ventral muscle bands. Visualization of pinned specimens was relatively simple, using volume-rendering and cropping techniques to examine internal detail.

The quality of scans for the two frozen specimens was equally detailed, albeit with the occasional complication of gut contents, which tend to be desiccated in pinned specimens. For scans of frozen bees it was necessary to create segments of musculature for rendering and display. Although slightly more time consuming, results from frozen specimens matched pinned specimens in detail.

Outgroup taxa were chosen across several groups, as some relationships among bee families remain unsettled. Several phylogenies have been proposed using morphological (Roig-Alsina and Michener, 1993; Alexander and Michener, 1995; Michener, 2007) as well as molecular methods (Danforth et al., 2013; Hedtke et al., 2013). As this study focused on questions of morphology, a more morphologically oriented approach was chosen (fig. 20, based on Michener, 2007). The selection of outgroup taxa, however, was distributed so as to encompass proposed phylogenies.

Several subfamilies and tribes were examined within Halictidae: one from Rophitinae, two from Dieunomiini (Nomiinae), and the remainder from Halictinae—two from Halictini, two from Caenohalictini, and three from Augochlorini (Halictinae). With good agreement between morphological and molecular studies regarding the phylogeny of Halictidae (Michener, 2007; Danforth et al., 2013), Rophitinae was chosen as the outgroup for comparisons within Halictidae.

RESULTS

OUTGROUP TAXA

Osmia (*Melanosmia*) *bucephala* Cresson (Megachilidae: Megachilinae: Osmiini) (fig. 5) has the typical arrangement of metasomal muscle morphology, as previously documented by Snodgrass (1956). In particular, both oblique and dorsal-lateral muscles are thin and planar with insertion areas approximately the same width as the muscle sheet. Attachments on the antecosta of T2 are similarly arranged.

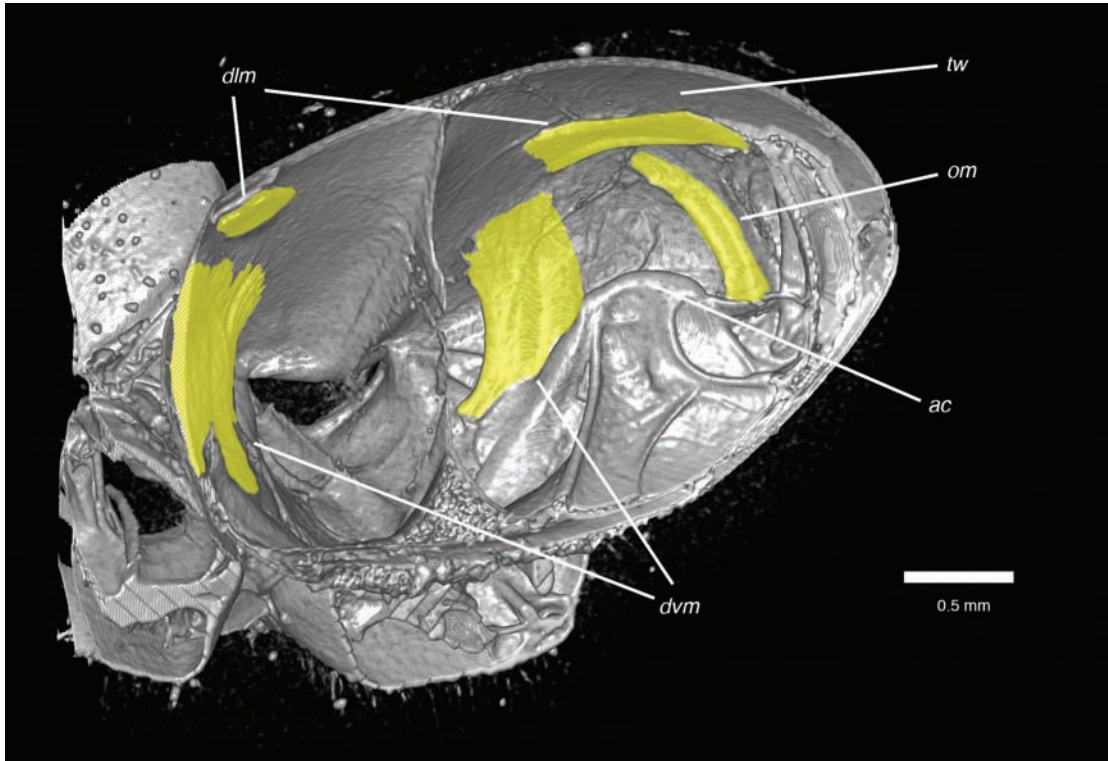


FIGURE 5. Cutaway view of *Osmia (Melanosmia) bucephala* Cresson (Megachilidae: Megachilinae: Osmiini), showing right *om*, *dvm*, and *dlm*. Also visible are left *dlm* and *dvm*. Left *dvm* cross-hatching is shown to denote thin morphology normally seen in metasomal muscle.

Protoxaea gloriosa (Fox) (Andrenidae: Oxaeinae) is shown in posterior view (fig. 6A) and lateral cutaway (fig. 6B). Dorsal-ventral origins are wide, but the muscles themselves are still very flat, as seen in the cross section. Oblique muscles are similarly sheetlike and planar, but approximately 1/4 the width of the dorsal-ventral muscles. Similar to *O. bucephala*, the dorsal-lateral muscles possess small origin and insertion areas, basically the same size as the muscle cross-sectional area.

Melipona grandis Guérin-Ménéville (Apidae: Apinae: Meliponini) (fig. 7) is an example of “abbreviated” metasomal muscle morphology. In particular, the dorsal-ventral muscles feature a low insertion point on T1, with the oblique muscles similarly arranged. The dorsal-lateral muscles are also short with small origin and insertion points.

Centris (Ptilotopus) americana (Klug) (Apidae: Apinae: Centridini) in posterior view (fig. 8A) highlights the positions of the dorsal-lateral muscles and oblique muscles. Both are also planar/sheetlike with small origin and insertion points. The dorsal-ventral muscles (fig. 8B) are wider with a larger origin area, but the muscles are flat, similar to other outgroup taxa.

Colletes thoracicus Smith (Colletidae: Colletinae) (fig. 9) has a broad dorsal-ventral muscle origin, but retains the planar structure seen in previous specimens. Dorsal-lateral muscles and oblique muscles are similarly broad but flat.

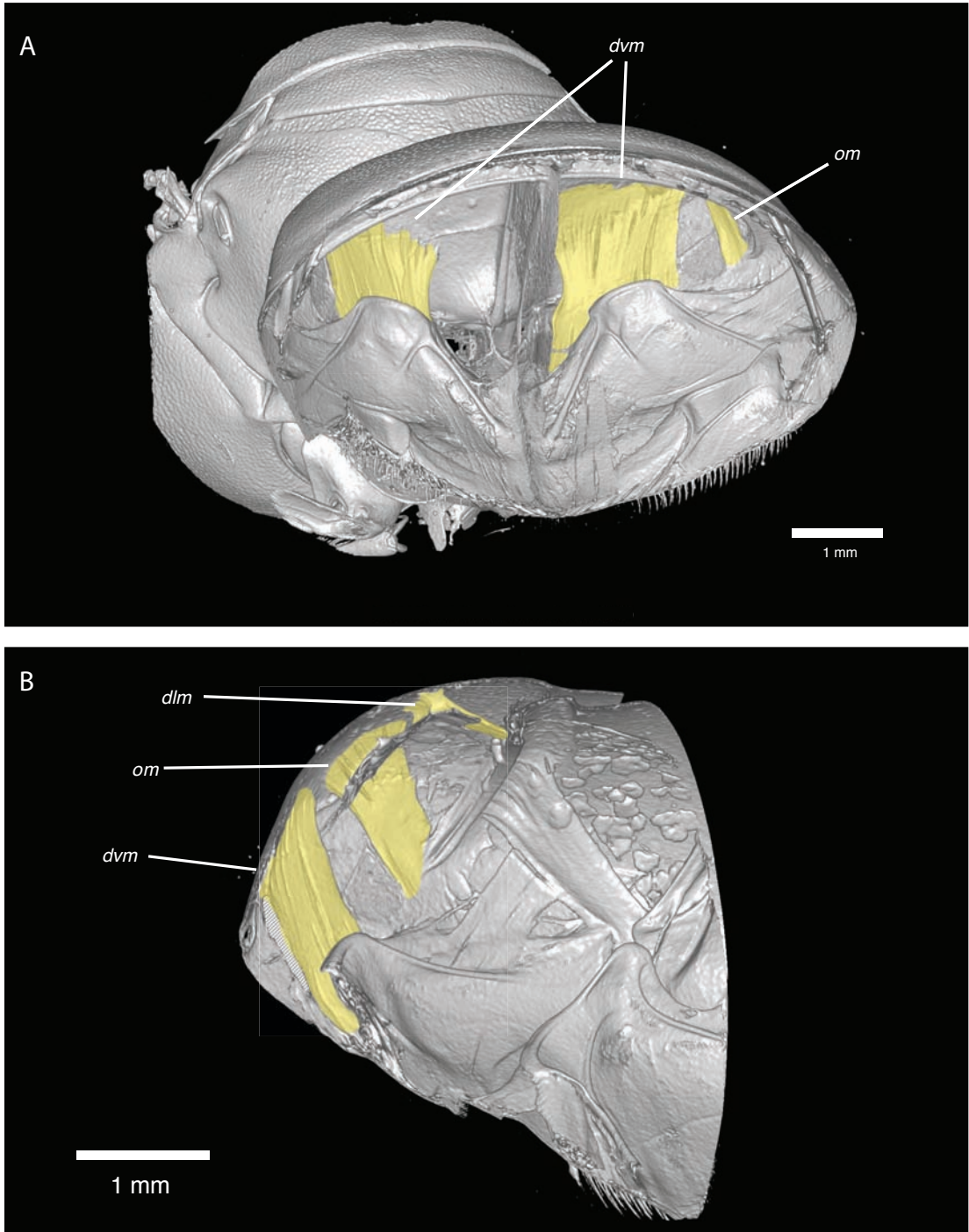


FIGURE 6. A. Cutaway view of *Protoxaea gloriosa* (Fox) (Andrenidae: Oxaeinae), looking from posterior toward thorax. Antecosta of the sternites partially blocks view of the *dvm*, but the wide bandlike morphology is clear. B. Lateral cutaway view displaying thin cross section of *dvm*.

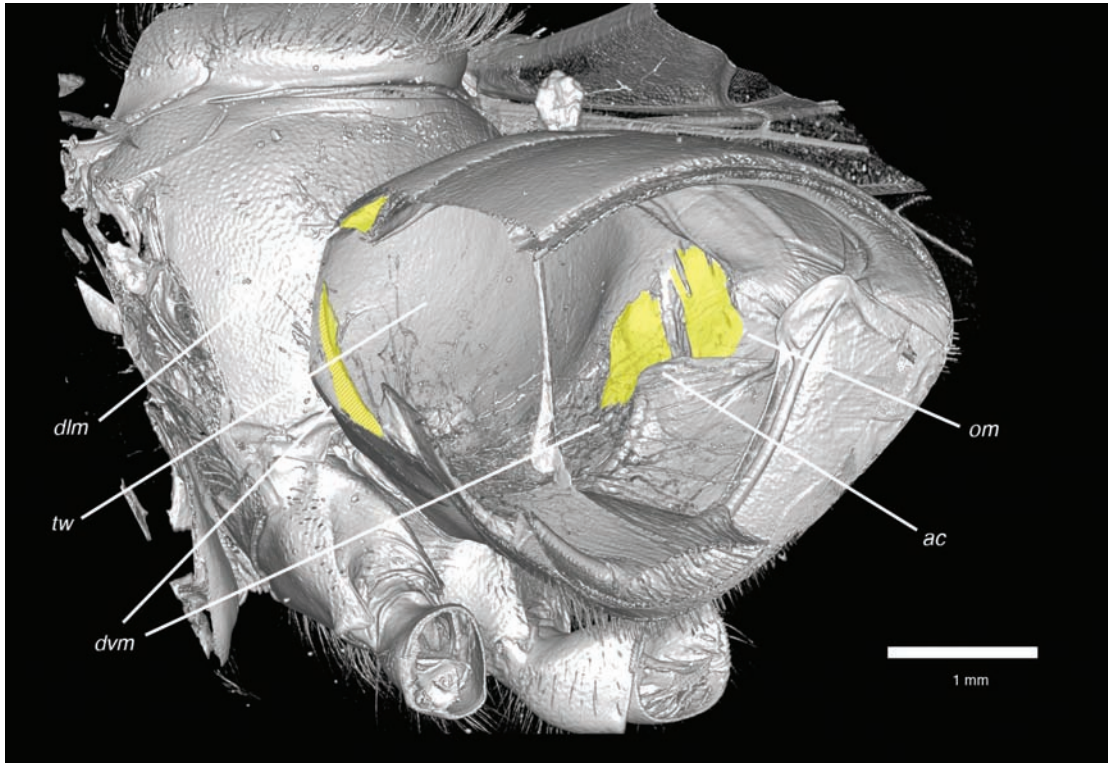
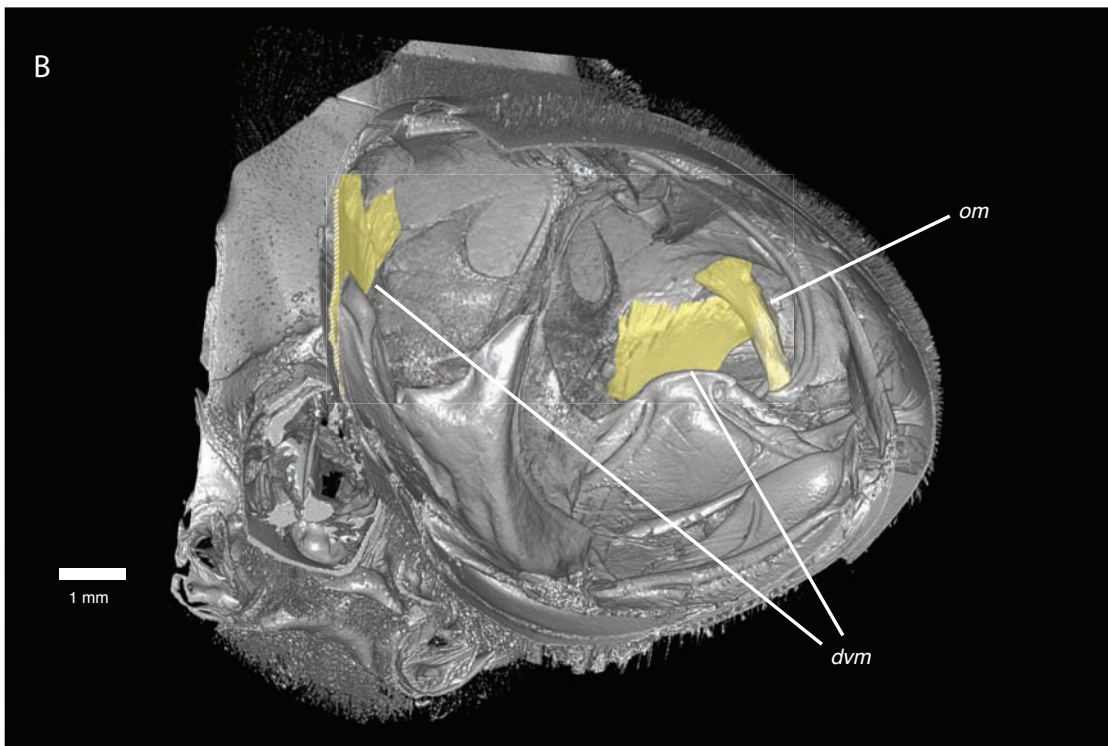
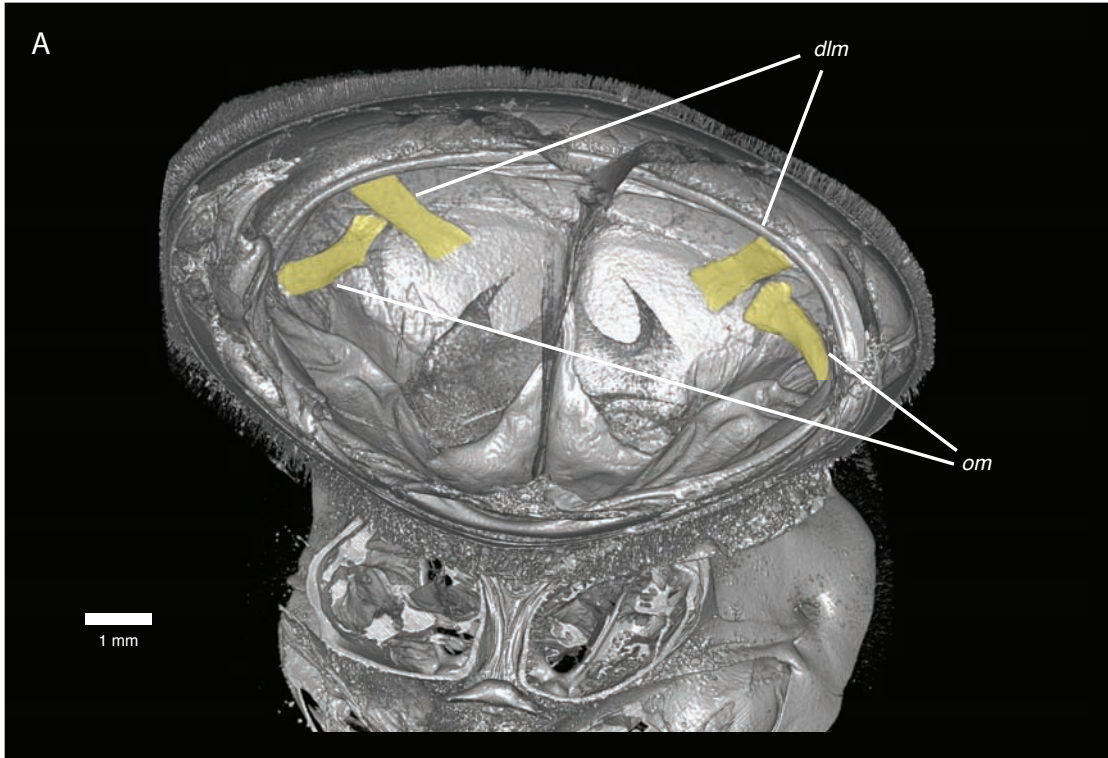


FIGURE 7. *Melipona grandis* Guérin-Méneville (Apidae: Apinae: Meliponini), lateral cutaway view, displaying all three muscle groups and *dvm* in cross section.

HALICTID TAXA

Xeralictus bicuspidariae Snelling and Stage (Rophitinae: Rophitini), the most basal sweat bee examined, features morphology similar to the outgroup species (fig. 18A), including a relatively planar dorsal-ventral muscle with a planar cross section (fig. 18B). A male specimen of *X. bicuspidariae* was also scanned, and displayed muscle morphology identical to the female.

Two specimens of the genus *Dieunomia* Cockerell (Nomiinae: Dieunomiini) were scanned. The dorsal-ventral muscle of *Dieunomia heteropoda kirbii* (Smith) (fig. 11A) is large and robust, separated from the tergal walls and bulging into air-sac space, with correspondingly large origin and insertion areas. The cross-sectional area is significantly larger and more circular than the planar abdominal muscles seen in the specimens of the outgroup taxa. While more planar and bandlike than the *dvm*, the oblique muscle (fig. 11B) has large origin and insertion areas. The striplike dorsal-lateral muscle is similar in structure to outgroup species. The origin of the dorsal-ventral muscle of *D. nevadensis arizonensis* (Cockerell) (fig. 12A) is high on the body wall, and the bundle of fibers expands through the air sac to the sternum below. The cross section (fig. 12B) is decidedly not planar or sheetlike. The oblique muscle and dorsal-lateral muscles are similar to those in *D. heteropoda kirbii*. A male of *D. heteropoda kirbii* was also scanned and showed muscle morphology identical to the female.



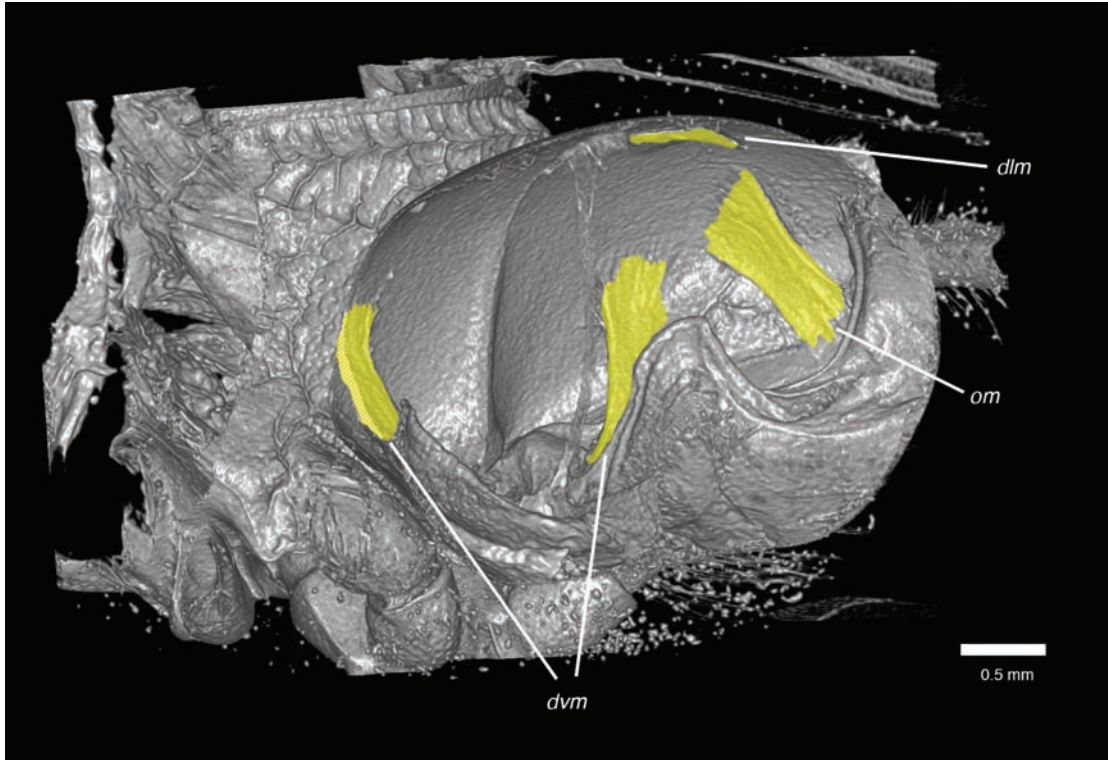


FIGURE 9. *Colletes thoracicus* Smith (Colletidae: Colletinae), lateral/posterior cutaway view, with cross section of *dvm*.

Lasioglossum (Dialictus) sp. (Halictinae: Halictini) (fig. 1) is shown in dorsal habitus, featuring the location of the metasomal air sacs. Visualization of the air sacs is achieved by rendering the integument as translucent and the air sac as a solid. A close examination of the rendered air sacs reveals apparent “grooves,” the imprints of the muscles. It can be seen that the depth of the grooves is greater than would be expected for simple thin-banded metasomal muscles. A volume-rendered cutaway (fig. 10) of the metasoma of *L. (Dialictus)* details muscle locations. The dorsal-ventral muscle is large and robust, separated from the tergal wall and expanded into the air-sac space. Likewise, while smaller than the dorsal-ventral muscle, the dorsal-lateral and oblique muscles, rather than thin, planar layers, are cylindrical bundles, extending away from the body wall.

Sphecodes albilabris Kirby (Halictinae: Halictini), a cleptoparasitic bee, was also scanned and displays morphology very similar to *L. (Dialictus)*, with large dorsal-ventral musculature, extending into the abdominal air sac.

Thrinchostoma (Eothrinchostoma) torridum (Smith) (Halictinae: Halictini) (fig. 17A), seen in a posterior lateral cutaway, also shows the large dorsal-ventral morphology seen in previous

FIGURE 8. A. *Centris (Ptilotopus) americana* (Klug) (Apidae: Apinae: Centridini), posterior cutaway view, showing thin band morphology of *om* and *dvm*. B. Lateral cutaway view, with cross section of *dvm*.

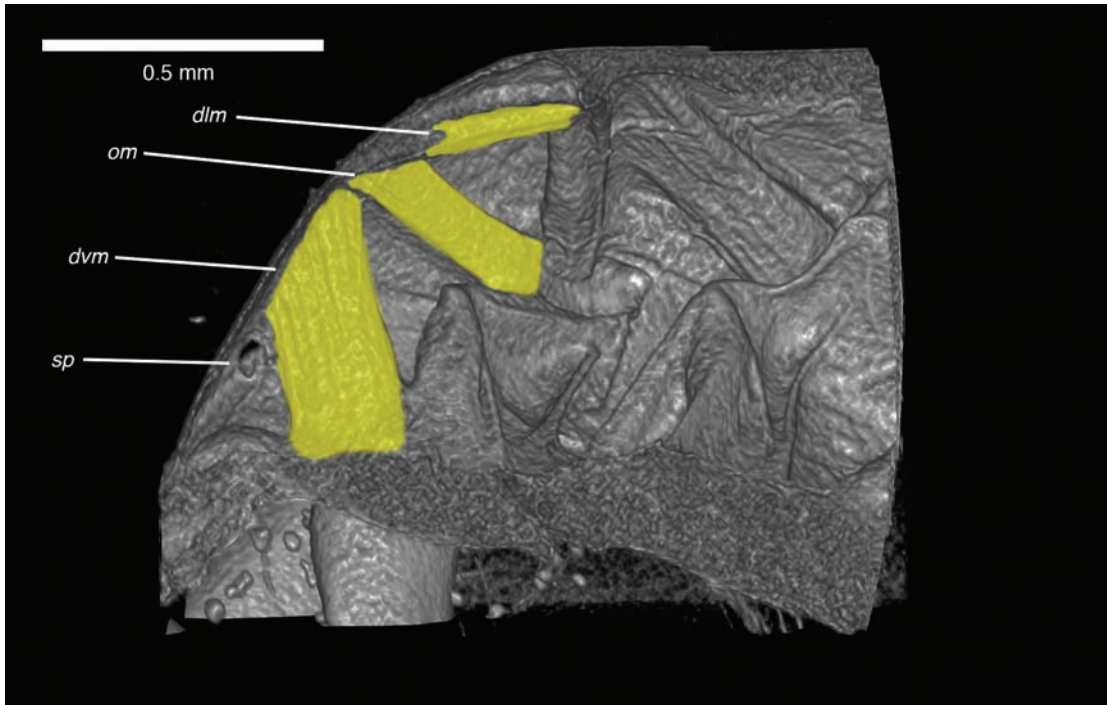


FIGURE 10. *Lasioglossum (Dialictus)* sp. (Halictinae: Halictini) lateral cutaway view.

species. The oblique muscle is cylindrical and separate from the T1 body wall. The dorsal-ventral muscle cross section is similarly subcircular (fig. 17B).

All three types or pairs of metasomal muscles are visible in a single view of *Caenohalictus rostraticeps* (Friese) (Halictinae: Caenohalictini) (fig. 14). With a large origin, the dorsal-ventral muscle is separated from the body wall and subcircular in cross section. The oblique muscle, while more planar, also has a large origin and is broader than previous species such as *A. aurata*. The dorsal-lateral muscle is thin and planar, similar to nonhalictids.

Pseudagapostemon (Pseudagapostemon) citricornis (Vachal) (Halictinae: Caenohalictini) (fig. 16A) also possesses large dorsal-ventral muscles with correspondingly large areas of origin. While not as large as the *dvm*, the oblique muscles are larger than the outgroup taxa and also extend into the air sac (fig. 16B). A male specimen of *P. citricornis* was also scanned, and displayed muscle morphology identical to the female.

Augochlora (Augochlora) pura (Say) (Halictinae: Augochlorini) (fig. 4) was the other frozen specimen scanned. Due to the distended gut, segmentation of the metasomal muscles was necessary for visualization. The dorsal-ventral muscles are cylindrical and separated from the body wall. The oblique muscles, while smaller than the *dvm*, are likewise more bundlelike than the outgroup specimens and expanded into the air sac space. The dorsal-lateral muscle is relatively bandlike, but not as planar as in outgroup taxa.

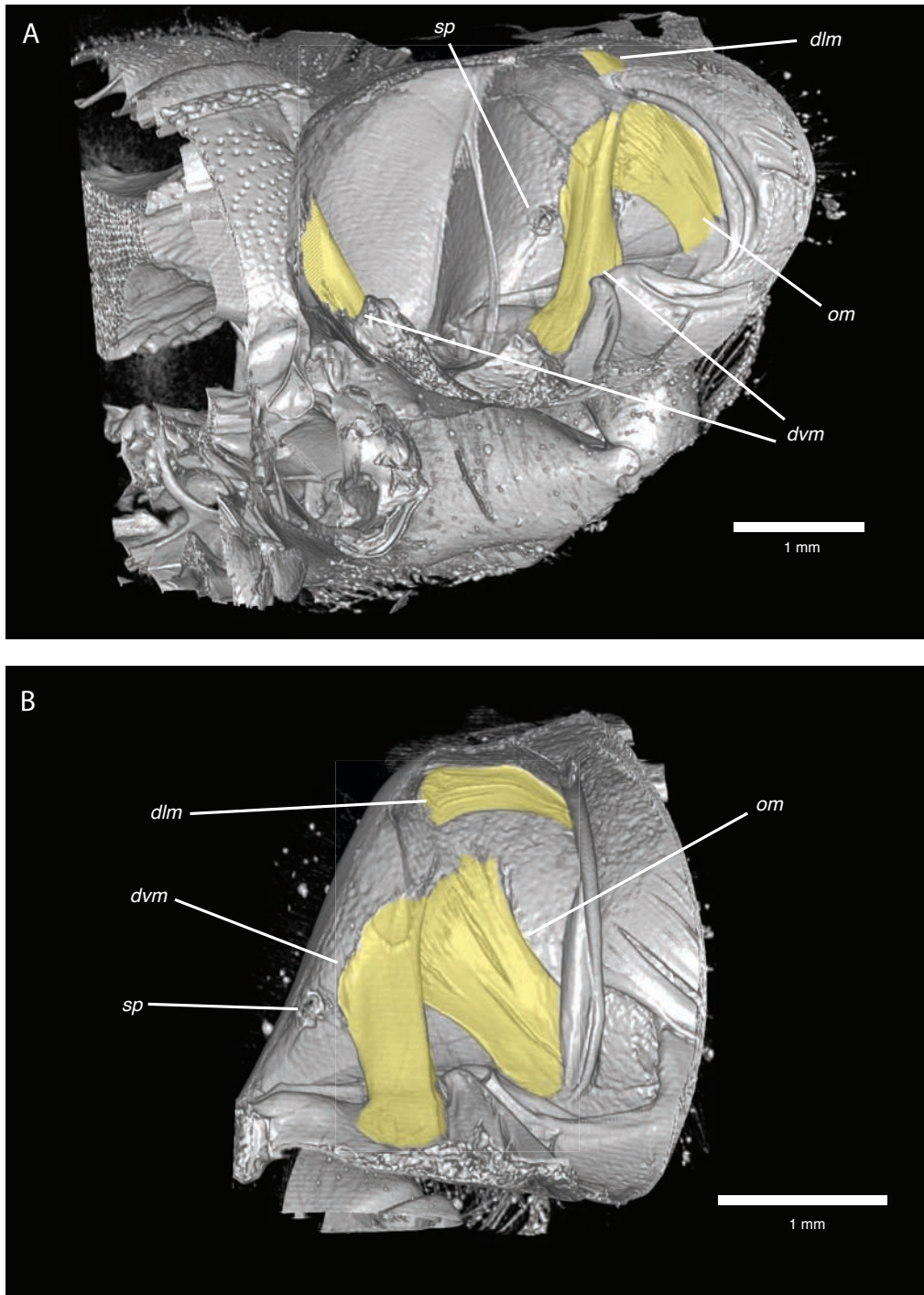


FIGURE 11. *Dieunomia heteropoda kirbii* (Smith) (Nomiinae: Dieunomiini): **A.** Lateral/posterior cutaway, with cross section of *dvm*. **B.** Lateral cutaway, showing separation of *dvm* from anterior metasomal body wall.

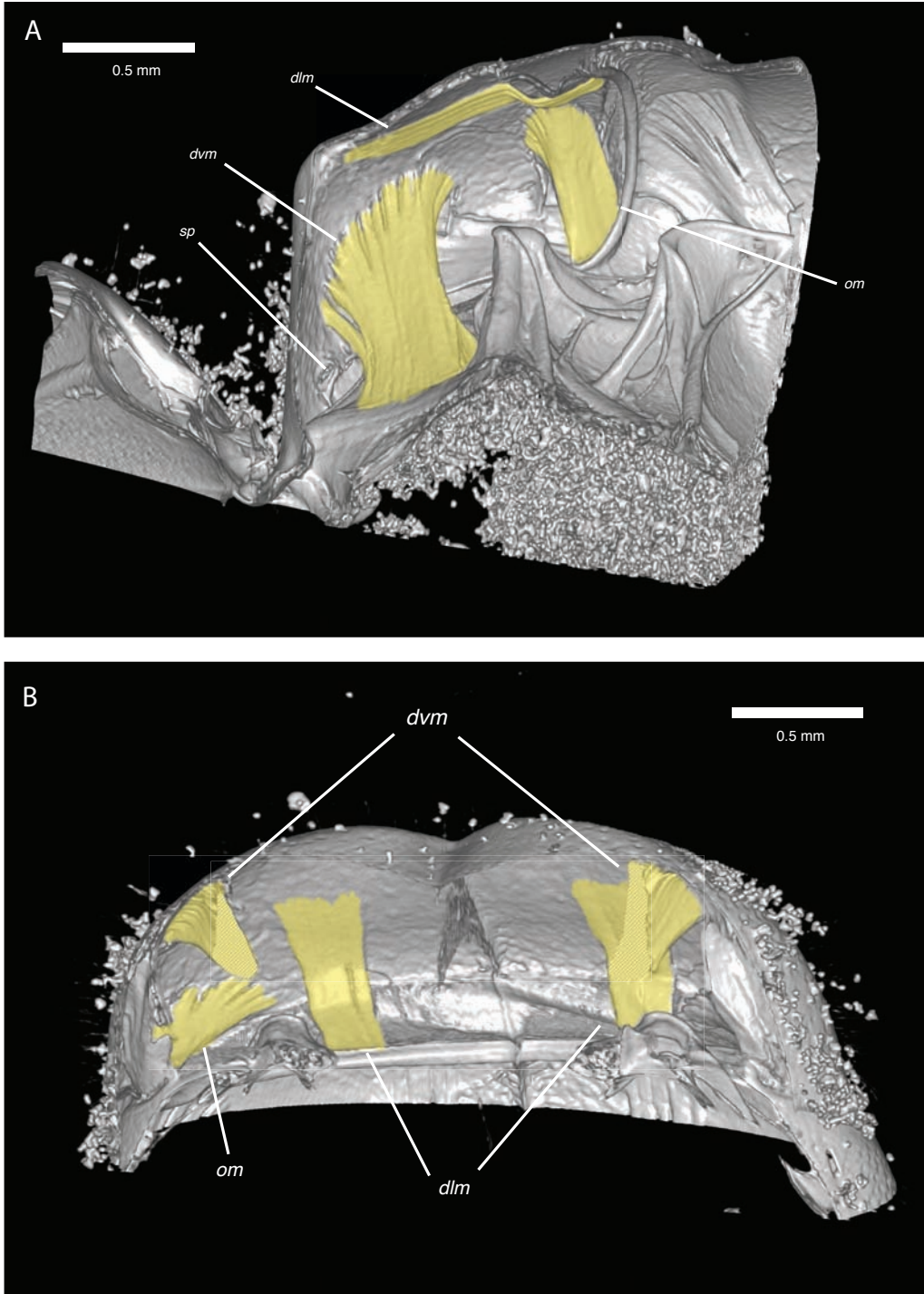


FIGURE 12. *Dieumonia nevadensis arizonensis* (Cockerell) (Nomiinae: Dieunomiini): **A.** lateral cutaway, showing large *dvm* in relation to striplike *dlm*. **B.** Ventral cutaway view, showing large cross section of *dvm*.

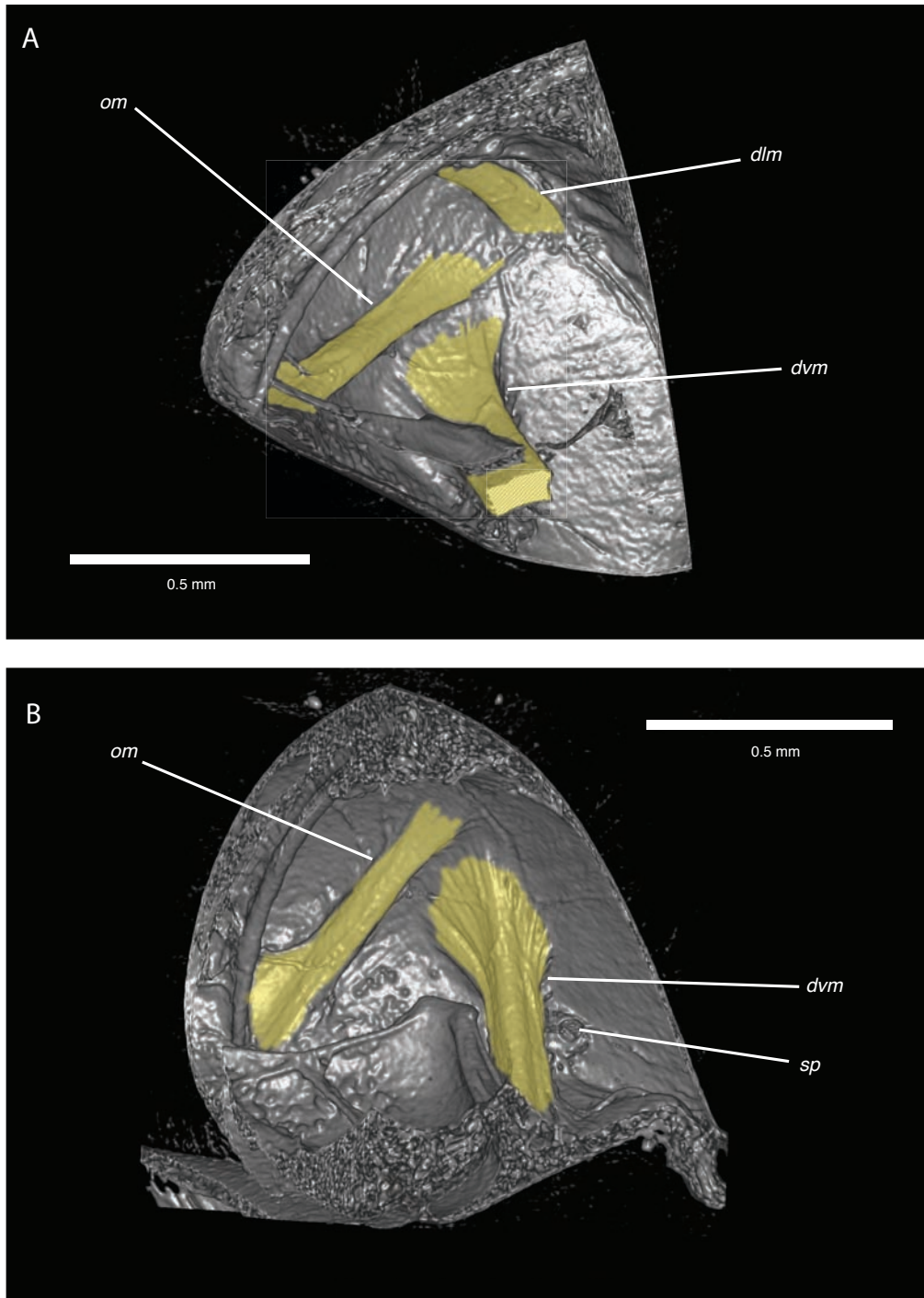


FIGURE 13. *Augochlorella aurata* (Smith) (Halictinae: Augochlorini): **A.** Posterior cutaway view, showing *dvm* origin and large cross section. **B.** Lateral cross section, showing large *dvm* insertion and separation from T1 body wall.

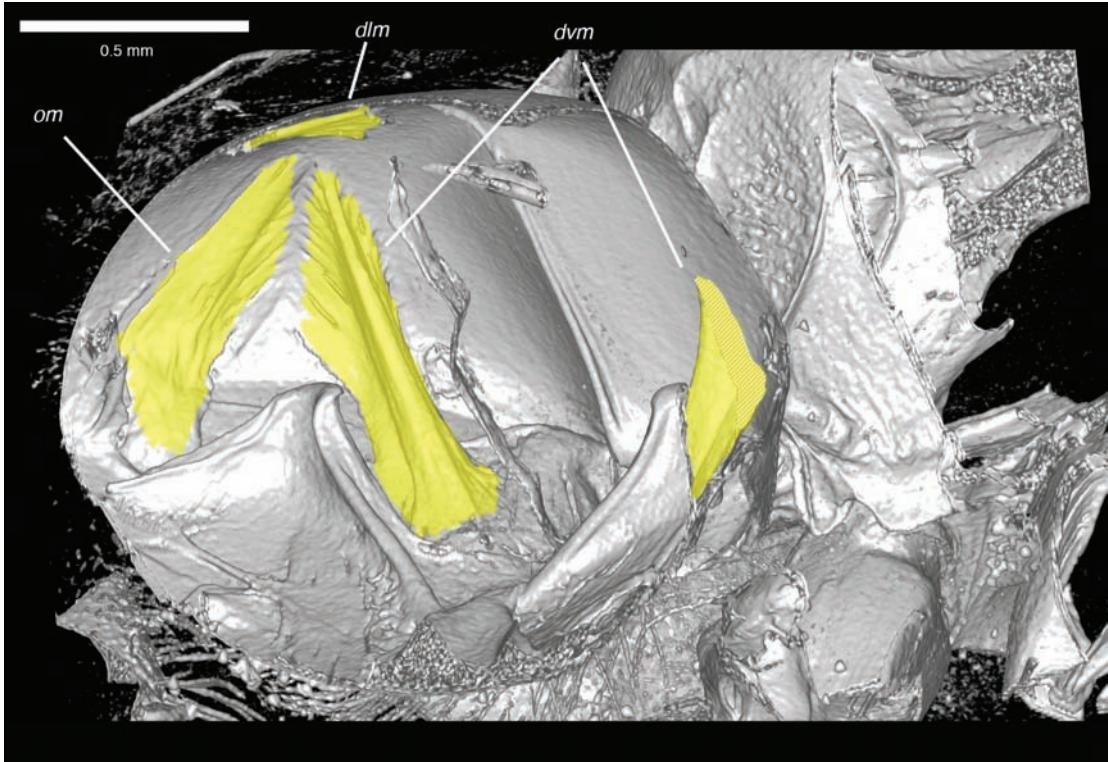


FIGURE 14. *Caenohalictus rostraticeps* (Friese) (Halictinae: Caenohalictini) in a posterior/lateral cutaway view, showing *dvm*, *dlm*, *om*, and T1 *sp*.

Augochlorella aurata (Smith) (Halictinae: Augochlorini) (fig. 13A) possesses the pattern of previous halictid specimens with a large cross-sectional *dvm*, expanded into the air sac with a large origin, high on the T1 body wall (fig. 13B). The oblique and dorsal-lateral muscles are more planar, similar to previous species.

A posterior cutaway view of *Megalopta* sp. (Halictinae: Augochlorini) also displays all three pairs/types of metasomal muscles (fig. 15A). The dorsal-ventral muscle cross section (fig. 15B) is more robust and bundlelike than in outgroup taxa, and extends into the air-sac space. This particular specimen displayed minor preservational artifacts, where the oblique muscle separated due to desiccation (fig. 15A). The clean break, however, still allowed identification.

DISCUSSION

Traditionally, Colletidae are considered a comparatively primitive lineage of bees (e.g., Michener, 1944, 2007). *Colletes thoracicus* (fig. 9) features muscle morphology typical of outgroup species: the dorsal-ventral muscle has a thin, planar cross section with small origin and insertion areas. Although the *dvm* is separated from the body wall in areas, it is relatively short, with its origin approximately halfway between the dorsalmost and ventralmost points of T1. Likewise, the oblique and dorsal-lateral muscles are similarly thin, with correspondingly small

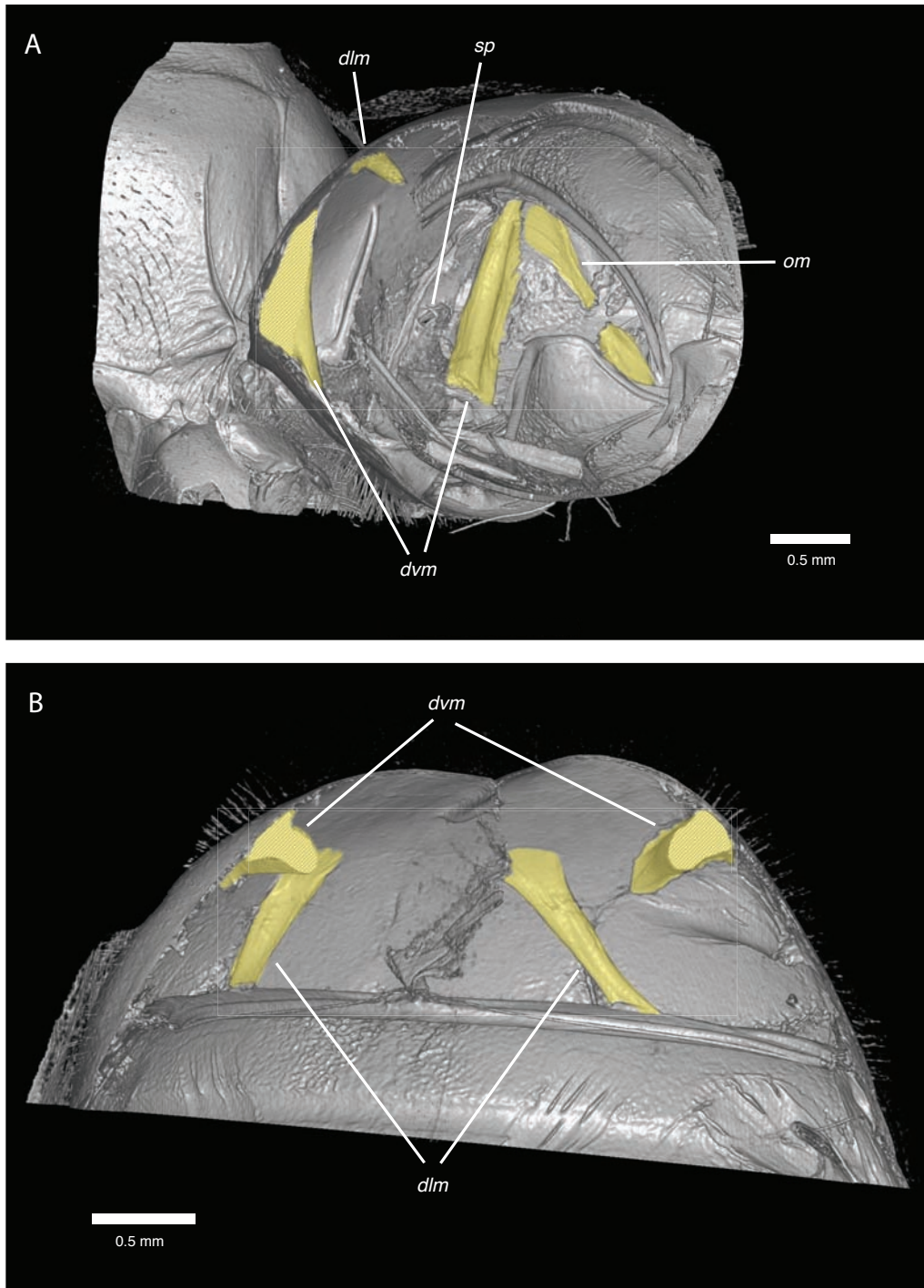


FIGURE 15. *Megalopta* sp. (Halictinae: Augochlorini): **A.** Posterior cutaway, showing large *dvm* cross section. Note fracture in oblique muscle due to desiccation. **B.** Ventral cutaway view, showing large *dvm* cross section.

origin and insertion areas. A comparison across all of the outgroup taxa with Halictidae reveals significant differences in metasomal muscle morphology (fig. 19). The outgroups feature relatively planar muscles, as noted for *C. thoracicus*, where each appears to be a thin layer of fibers, as is commonly seen in abdominal skeletal musculature (Chapman, 2012; Snodgrass, 1935). However, Halictidae all feature larger, more robust musculature. In particular, the proportions of dorsal-ventral muscle (*dvm*) resembles that of thoracic dorsal-ventral flight muscle, comprised of many fibers bundled into a cylindrical/subcylindrical structure. The *dvm* extends into the metasomal air sac, and in some specimens it is even separated from the body wall.

SUBFAMILY ROPHITINAE SCHENCK

TRIBE ROPHITINI SCHENCK S.L.

The subfamily Rophitinae is generally recognized as the earliest diverging among extant lineages of Halictidae (e.g., Alexander and Michener, 1995). Following Engel (2001, unpublished data), we recognize two tribes: Penapini and Rophitini, the latter of which was represented in the present work by *X. bicuspidariae*. Interestingly, *X. bicuspidariae* (fig. 18) possesses muscle morphology similar to nonhalictid species, with a planar *dvm* lying against along the T1 body wall. Although the *dvm* is thicker and more robust than in non-Halictidae, it is decidedly sheetlike, similar in form to the outgroup taxa. In addition, the *dml* is similarly broad and robust, but also lying against the body wall, along with the *om*.

SUBFAMILY NOMIINAE ROBERTSON

TRIBE DIEUNOMIINI ENGEL

Two specimens of the nomiine tribe Dieunomiini and the genus *Dieunomia* (*nevadensis arizonensis* and *heteropoda kirbii*) were examined and showed some surprising diversity. The *dml* is nearly identical in form, although the insertion point for the *om* is positioned more anteriorly in *D. heteropoda kirbii* than for *D. nevadensis arizonensis*. *Dieunomia heteropoda kirbii*'s *om* is also much broader, with a large, fanlike origin, much closer in form to *D. nevadensis arizonensis*'s *dvm*. Likewise, while both specimens possess large *dvms*, *D. nevadensis arizonensis* features a much larger *dvm* point of insertion and overall size than in *D. heteropoda kirbii*.

SUBFAMILY HALICTINAE THOMSON

TRIBE HALICTINI THOMSON

Three species in tribe Halictini were examined: *Lasioglossum* (*Dialictus*) sp. (figs. 1, 10), *Sphecodes albilabris*, and *Thrinchostoma torridum* (fig. 17). *Lasioglossum* (*Dialictus*) sp. was the only fresh specimen examined. In each example, the *dvm* has a round cross section, is separated from the body wall, and features a large area of insertion, typically closer to the ventralmost points of T1 than halfway between the ventralmost and dorsalmost points of T1. The

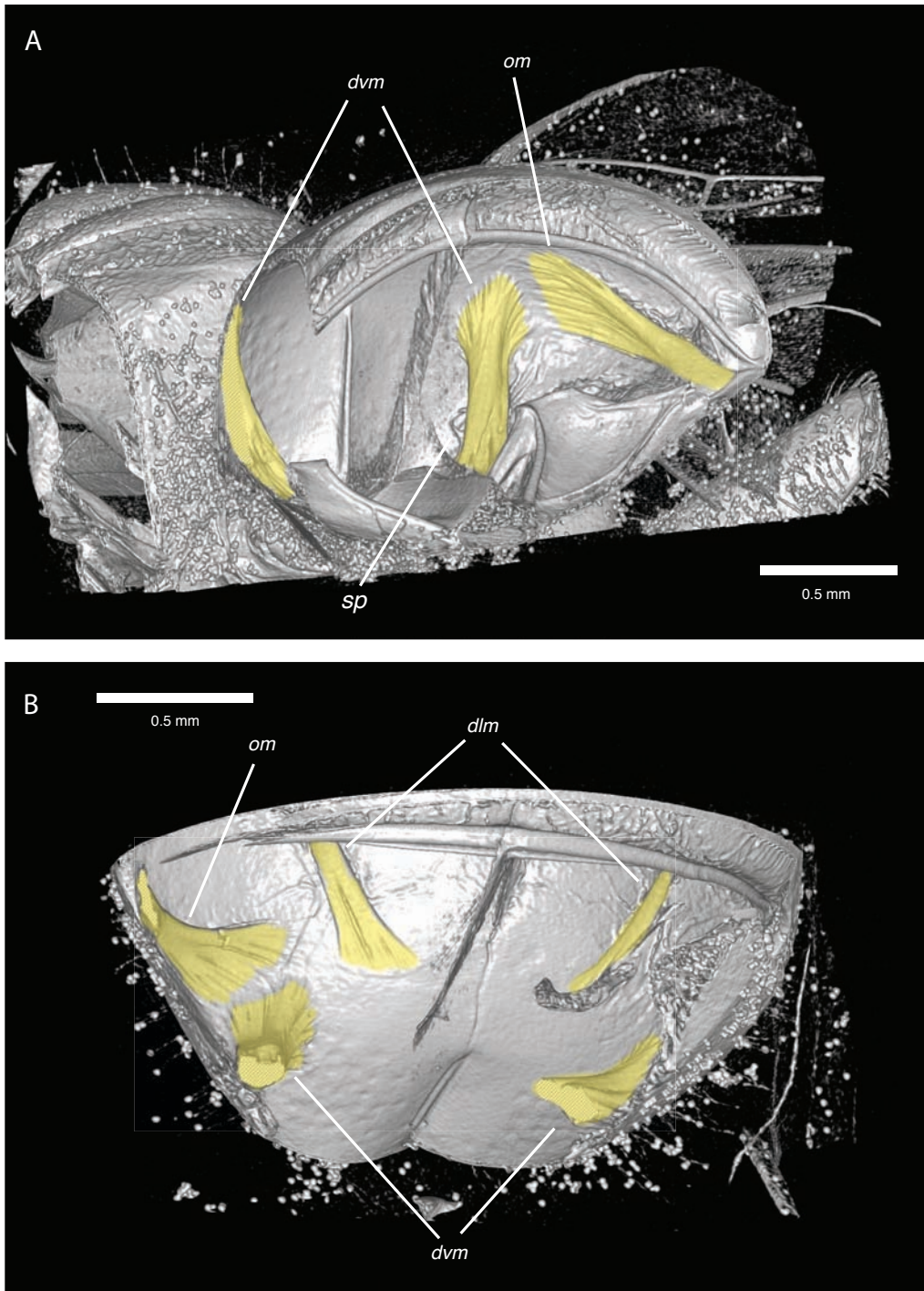


FIGURE 16. *Pseudagapostemon* (*Pseudagapostemon*) *citricornis* (Vachal) (Halictinae: Caenohalictini): A. posterior cutaway, showing *dvm* with cross section and *om*. Note spiracle (*sp*) on T1 anterior. B. Ventral cutaway view, showing locations of all 3 T1 metasomal muscles. Note large *dvm* and *om* origin areas and *dvm* cross section.

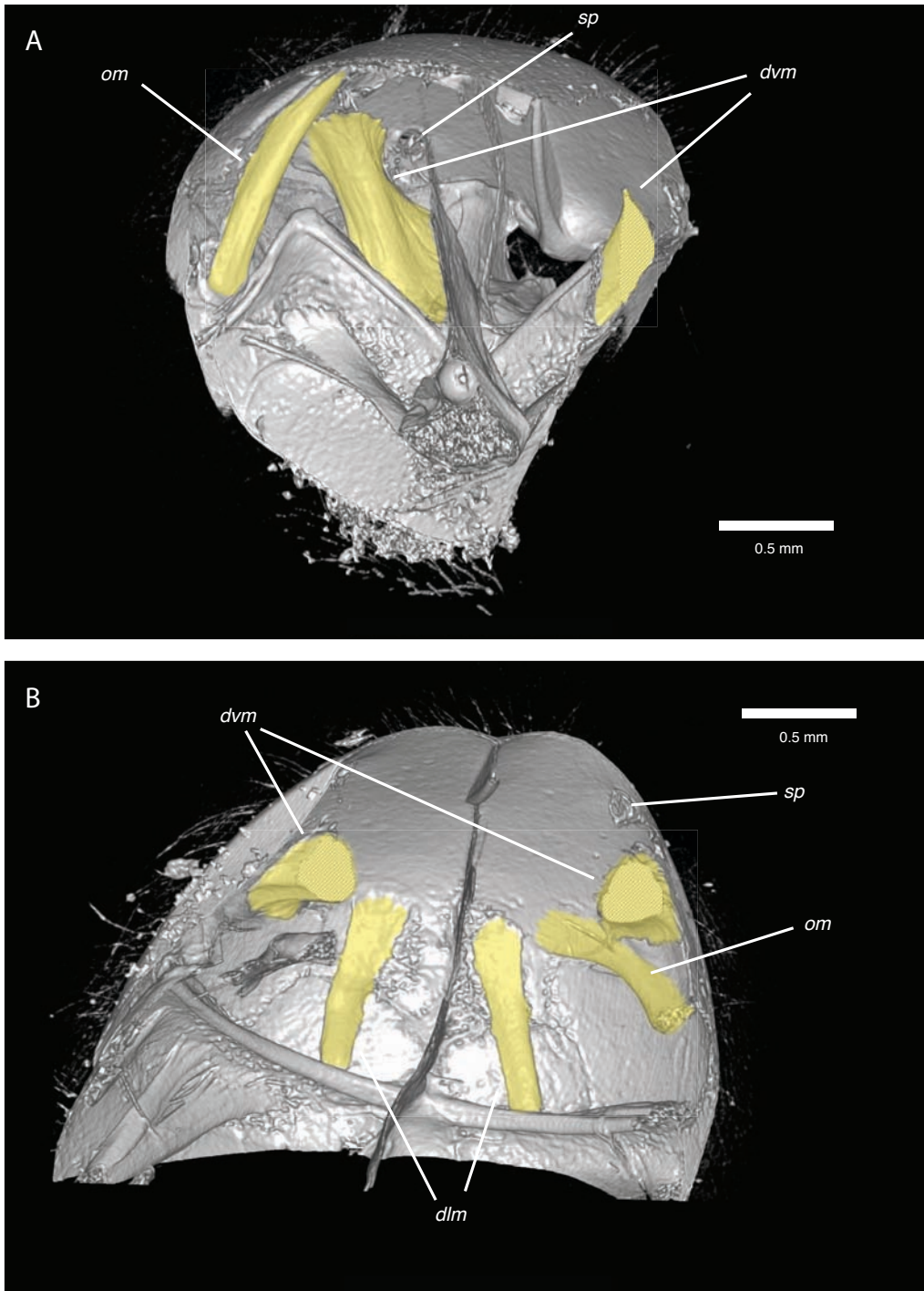


FIGURE 17. *Thrinchostoma torridum* (Smith) (Halictinae: Halictini): **A.** Posterior/lateral cutaway, showing cross section of *dvm*. T1 *sp* and bundlelike *om* also visible. **B.** Ventral cutaway view, showing large cross section of *dvm*.

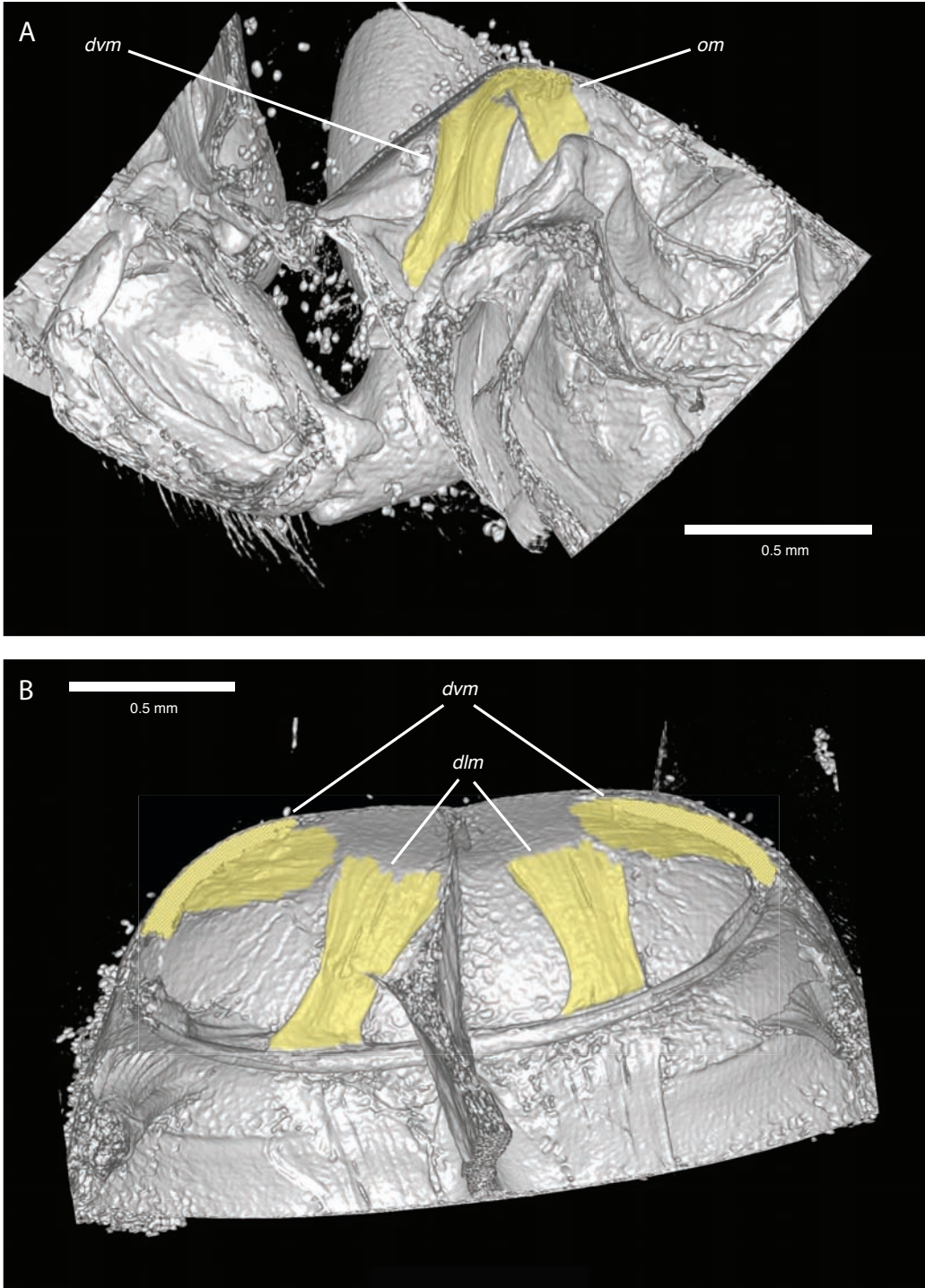


FIGURE 18. *Xeralictus bicuspidariae* Snelling and Stage (Rophitinae: Rophitini): **A.** Lateral cutaway, showing *dvm* and *om* in profile. **B.** Ventral cutaway, showing comparatively thin *dvm*.

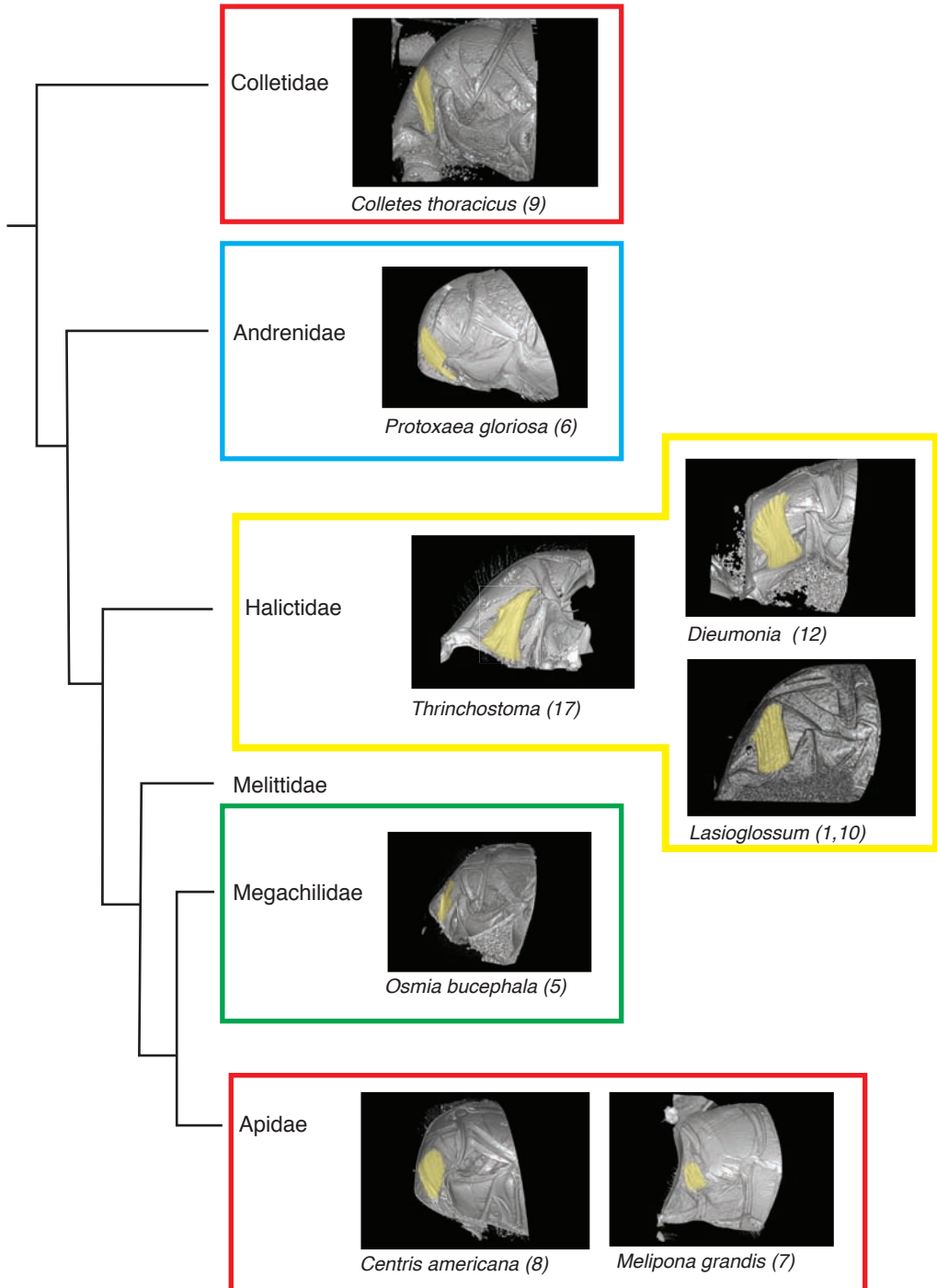


FIGURE 19. Simplified phylogeny of selected bee families, based on Michener (2007), with distribution of outgroup taxa selected for study. Dorsal-ventral muscle is highlighted to show differences across families and similarity within Halictidae. Numbers in parenthesis next to taxon names correspond to figure numbers of detailed images.

oblique muscles, while not as robust as the *dvm*, are larger and more expanded into the air-sac space than in *C. thoracicus*.

Thrinchostoma has broad *om* insertion areas, a round cross section, and they are surrounded by air-sac space for some of their length. *Lasioglossum* (*Dialictus*) and *S. albilabris* are very similar, with robust *om*, although situated along the T1 lateral body walls.

TRIBE CAENOHALICTINI MICHENER

Similar to *Thrinchostoma*, *Pseudagapostemon* has a broad *om* insertion area, a round cross section, and is surrounded by air-sac space for some of its length. *Caenohalictus*, however, has an *om* that more resembles non-Halictidae outgroup taxa.

TRIBE AUGOCHLORINI BEEBE

Three species of Augochlorini were scanned: *Augochlorella aurata* (fig. 13), *Augochlora pura* (fig. 4), and *Megalopta* sp. (fig. 15). Of the three, *A. pura* was the only fresh specimen examined. As with Halictini and Caenohalictini, all specimens possessed a large, cylindrical *dvm* with a large muscle origin high on the body wall of T1. The *dvm* is separated from the body wall for some length, completely surrounded by the air-sac space.

Oblique muscles for Augochlorini appear to be more consistent across examined taxa. Smaller than the *dvm*, the *om* is somewhat planar yet thick, with origin and insertion points not significantly larger across than the muscle itself.

Dorsal-lateral muscles show some variation, with *A. pura* and *A. aurata* possessing short but somewhat broad muscles, and *Megalopta* sp. having a more cylindrical form. The *om* for *Megalopta* sp. also appears to separate from the body wall for a short section.

ENLARGED MUSCLES IN HALICTIDAE

Ten of the 11 halictid species examined featured unusual metasomal musculature, especially in the *dvm*, with large, cylindrical bundles of muscles often extending away from the body wall and surrounded by air-sac space. This muscle structure is similar to that of the thoracic flight apparatus—the dorsal-ventral and dorsal-lateral thoracic muscles—serving to power wing motion.

FUTURE DIRECTIONS

This study has concentrated on the morphology of the unusually large muscles, especially the dorsal-ventral muscle, found within T1 of Halictidae and the unique form of these in the Nomiinae and Halictinae. Indeed, the shared unique form of the *dvm* in Nomiinae and Halictinae is likely a further synapomorphy supporting this clade within Halictidae. A logical next step following comparative anatomy is to determine the function, in this case, of the enlarged three pairs of muscle groups in Halictidae. These specific muscles do not appear to be tied to any specialized movements unique to pollen collection as they are present in both males and females (where observed), as well as in *S. albilabris*. While it is possible that they are merely for abdominal com-

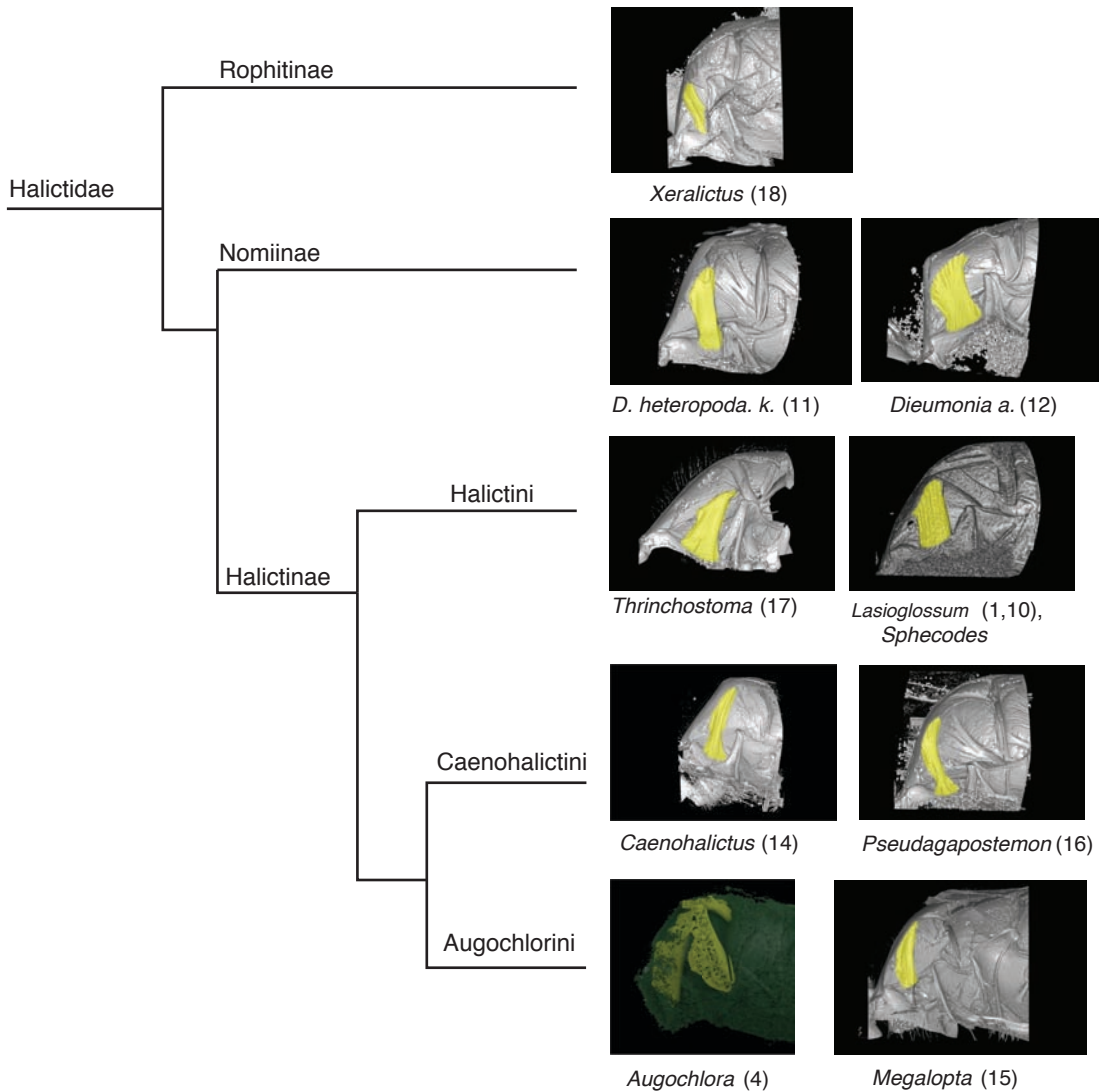


FIGURE 20. Phylogeny of the Halictidae, based on Michener (2007). Dorsal-ventral muscle is highlighted to show similarities across Halictidae. Numbers in parenthesis correspond to figure numbers of detailed images.

pression, such as in abdominal pumping, the proportions of the muscles are so similar to thoracic flight muscles that it suggests they might be fibrillar in nature. A number of approaches are available for determining the function of these enlarged muscles. Previous studies have inserted electrodes into thoracic muscles to record neural impulses (Esch and Goller, 1991; King et al., 1996). These methods were used to determine the “indirect” properties of flight muscle—a single nerve impulse into a fibrillar muscle will cause it to “ring” for many activation cycles. Similar methods used on the halictid T1 muscles might indicate if they act in the same manner.

Insect flight muscle also uses tremendous amounts of energy, powered by a dense concentration of mitochondria. Electron microscopic studies have estimated that as much as 40% of the

volume of a flight-muscle cell in insects is taken up by mitochondria (Chapman, 2012). To date, there have been no large-scale studies of insect muscle at the cellular level across families of bees. Such a study would assess the metabolic activity of these metasomal muscle groups, though neural impulses would be the definitive test as to whether these muscles are fibrillar or not.

A question unasked thus far is “why?” Abdominal pumping has been shown to be involved in respiration for flight (Bailey, 1954), although when typically observed, the dorsal-lateral muscles are presumably the most active group for axial contraction of the abdomen. It is not clear to what degree tergo-sternal compression is involved in abdominal pumping. In addition, presumably all bees participate in some degree of abdominal pumping, but not all bees have enlarged muscles—the thin, planar muscles must serve large numbers of bees perfectly well, so what’s the reason for large muscles in Nomiinae and Halictinae?

Another possible, but not exclusive, explanation is sound. If the metasomal T1 muscles fibrillate, the enveloping air sac could serve as a resonance chamber. This has the potential for producing sound, but the vibrations could also possibly be for some other function.

Targeted field observations may help in uncovering more information regarding metasomal muscle function, but another possible application of μ CT is live scanning. Applied to the blowfly flight motor in a synchrotron setting, this technique has achieved spectacular results (Walker et al., 2014), although the equipment requirements are substantial. It may be possible to achieve useful results using a similar technique in a μ CT scanner in “live” mode. Two-dimensional projections could be used to observe the movement of sclerotized structures during abdominal pumping to determine relative segment motion in real time. This would operate similar to an old-fashioned “fluoroscope” and, while not providing 3D datasets, could shed some light on the issue.

Regardless of the functionality or purpose of these large muscle groups, an exploratory study such as this has shown there is a large amount of information that can be learned by looking beneath the exoskeleton of many insects. Previously unobserved, new insights into morphology, physiology, behavior, and phylogeny will doubtless be uncovered in the coming years, thanks to the application of new technologies.

ACKNOWLEDGMENTS

We are grateful to Jerome G. Rozen, Jr., for providing access to dried specimens in the AMNH collection. The 3D Slicer Development Community, in particular Andras Lasso, Steve Pieper, Csaba Pinter, and Jean Christophe Fillion Robin, was helpful with the segmentation software, even adding suggested features in a matter of hours. We also acknowledge the anonymous reviewers, whose comments and suggestions helped to improve the manuscript.

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