Using Micro-Computed Tomography to Study Larval Development in a Ground-Nesting Solitary Bee

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Big-Bee

Introduction

Solitary bees make up the majority of bee diversity, yet many ground-nesting species remain poorly studied because their development is difficult to observe within soil. Ground-nesting bees develop inside sealed subterranean brood cells, limiting observation without destructive sampling techniques that often result in brood mortality thereby preventing longitudinal data collection.[3,5] Micro-computed tomography (micro-CT) provides a non-destructive method for monitoring living subterranean brood cells over time in captivity. Here, we assess the feasibility of using micro-CT to measure larval position, body size, survival, development, and brood-cell architecture, with the broader goal of monitoring overwintering strategies in the ground-nesting bee *Melissodes tepidus timberlaki* (Apidae).

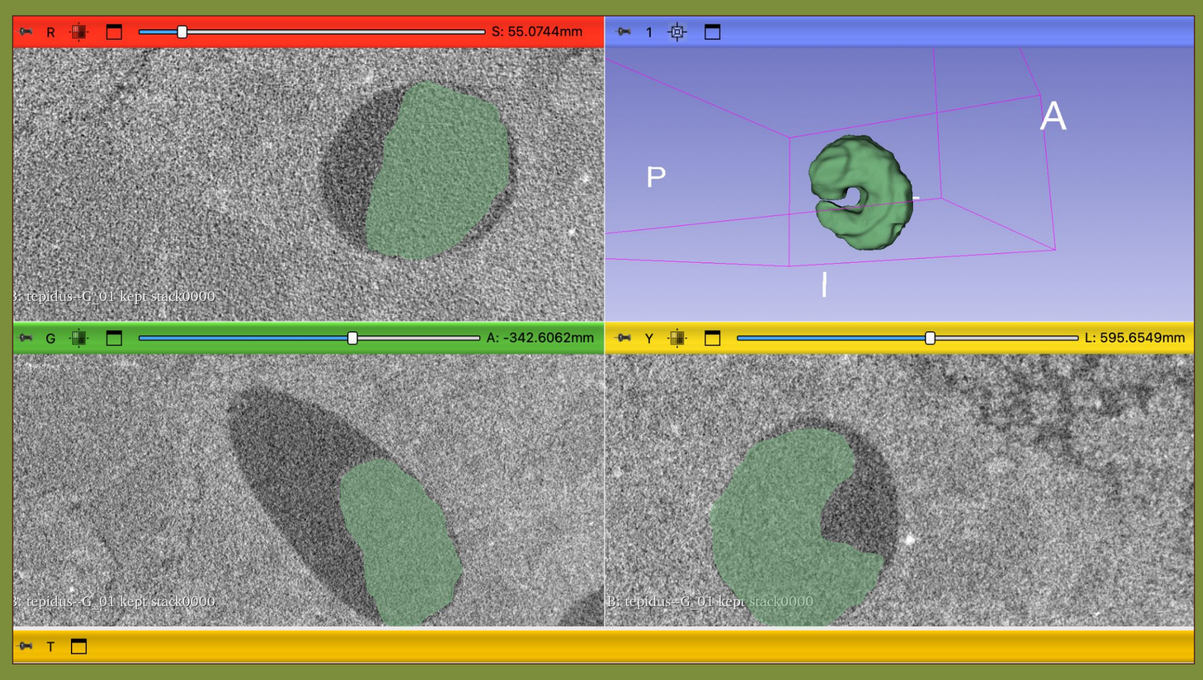

Fig. 1. Representative microCT segmentation and 3D reconstruction of a *Melissodes tepidus timberlaki* larva within soil substrate

Methods

Brood cells, along with the surrounding substrate, were excavated at the same time during the late-spring overwintering period from the ground-nesting bee *Melissodes tepidus timberlaki* (Cockerell, 1926). Therefore, all larvae assessed in this study were presumed to represent the same developmental stage. Brood cells were scanned using micro-computed tomography (micro-CT), and the resulting scans were acquired as TIFF image stacks. Each stack consisted of approximately 1,600 images per scan. Scan duration was kept under 8 minutes to minimize potential adverse effects of ionizing radiation on the brood cells. Scans were processed in Fiji version 1.54 and segmented in 3D Slicer version 5.10.[1,4]. Processing included cropping and contrast adjustment to improve segmentation and visualization of larval bodies. Larvae were digitally segmented from the surrounding substrate using automated thresholding techniques, although manual refinement and smoothing were required for each scan to obtain results. The 3D renderings were used to quantify the surface area and volume of each larva.

Results

Five intact larvae encased in soil substrate were successfully visualized and quantified using micro-CT without destructive sampling. Three-dimensional segmentation and rendering allowed reconstruction of larval morphology from TIFF image stacks (Fig. 1). Quantifiable 3D reconstruction was complicated by substrate interference, as larval tissue and the surrounding substrate frequently exhibited similar densities. Manual segmentation, contrast adjustment, and smoothing substantially improved the isolation of larvae from the surrounding substrate. Quantitative measurements, including volume and surface area, were successfully extracted from the segmented 3D models. Larval volume ranged from 59.7 to 110.0 mm^3, with a median of 70.3 mm^3 and an interquartile range of 17.7 mm^3(Fig. 2). Larval surface area ranged from 92.7 to 138.4 mm^2, with a median of 107.9 mm^2 and an interquartile range of 20.7 mm^2 (Fig. 2).
Surface-area-to-volume ratios ranged from 1.26 to 1.56 mm^{-1}, providing an additional measure of larval shape variation. Because only five larvae were imaged, these values are presented as preliminary descriptive measurements rather than estimates of population-level variation.

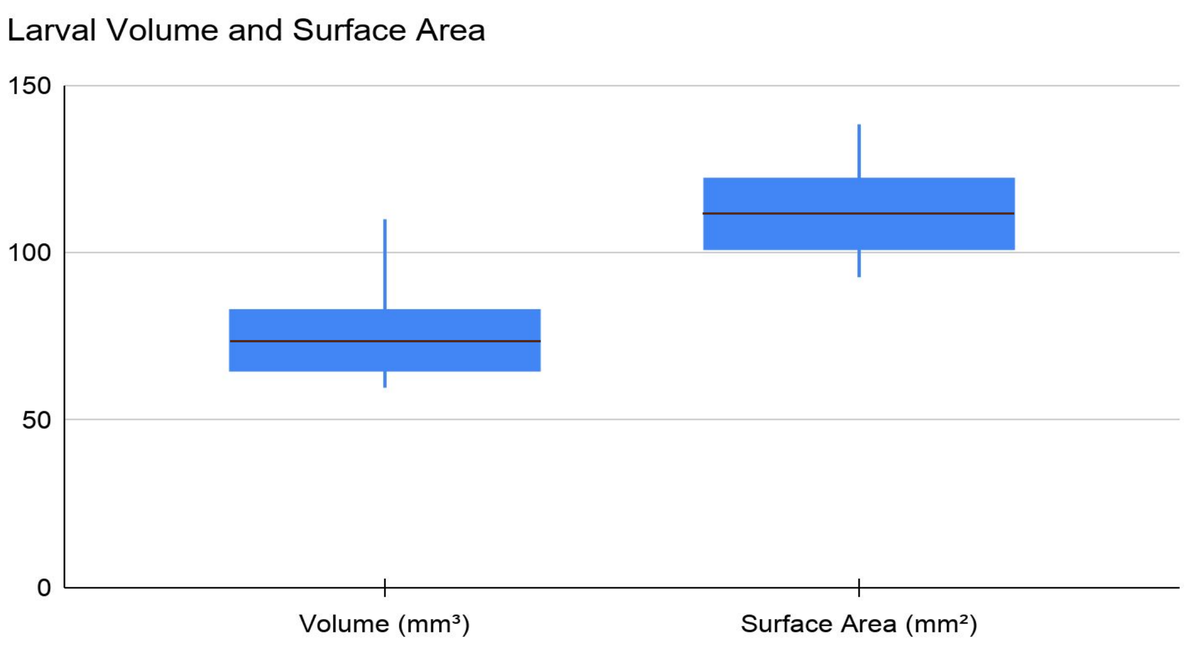

Fig. 2. Box plot showing volume and surface area measurements extracted from segmented 3D reconstructions (n=5)

Discussion

Micro-CT imaging is a feasible method for the non-destructive visualization and quantification of living *Melissodes tepidus timberlakei* larvae within sealed subterranean brood cells. This technique preserves brood-cell integrity, allowing longitudinal study of larval development and overwintering strategies in ground-nesting solitary bees. Previous studies have shown that soil conditions can strongly influence the emergence and survival of ground-nesting bees, demonstrating the importance of non-destructive approaches for studying overwintering development.[2] Despite successful reconstruction and quantification, segmentation was limited by substrate interference. Automated segmentation alone was insufficient, and manual contrast adjustment, segmentation refinement, and smoothing were required to obtain accurate, quantifiable 3D renderings. Future work may improve reconstruction efficiency through machine-learning-assisted segmentation approaches.

Literature Cited

[1]Fedorov, A., Beichel, R., Kalpathy-Cramer, J., Finet, J., Fillion-Robin, J. C., Pujol, S., Bauer, C., Jennings, D., Fennessy, F. M., Sonka, M., Buatti, J., Aylward, S. R., Miller, J. V., Pieper, S., & Kikinis, R. (2012). 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magnetic Resonance Imaging*, *30*(9), 1323–1341.

[2]Litz, A., Mesler, M., & Iler, A. (2025). Soil conditions and the emergence phenology and survival of a solitary, ground-nesting bee in a coastal dune environment. Ecological Entomology, 50(3), 514-522.

[3]Ordway, E. (1984). Aspects of the nesting behavior and nest structure of *Diadasia opuntiae* Ckll.(Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society, 216-230.

[4]Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682.

[5]Triplett, D. C., & Gittins, A. R. (1988). Nesting, mating and foraging habits of *Melissodes (Melissodes) tepida tepida* Cresson in Idaho (Hymenoptera: Anthophoridae).

Acknowledgements

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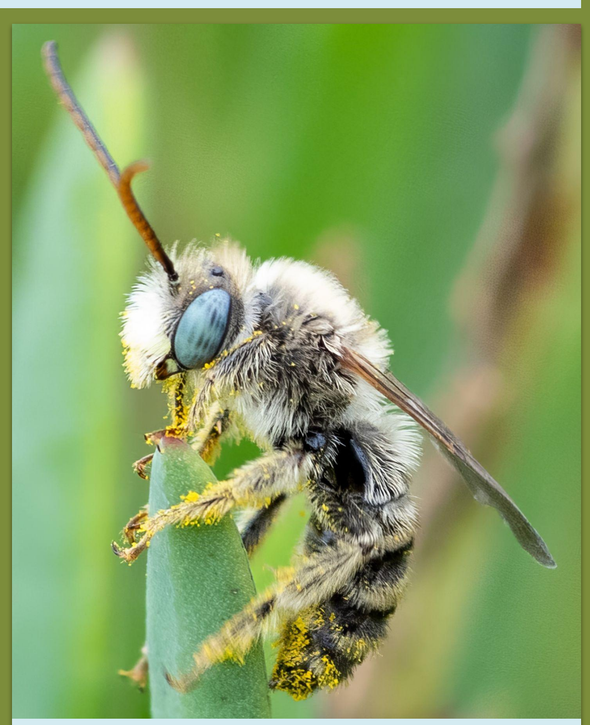

Melissodes tepidus timberlaki