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Three-Dimensional Printing of Agriculturally Important Mites Generated from Confocal Microscopy

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Recently, 3D printing has become an invaluable tool, now widely used for a broad range of applications. However, one of the major limitations of 3D printing is the difficulty in creating morphologically accurate models of complex biological specimens, which would otherwise require a highly skilled artist months to create. Here we present a new rapid method for generating highly detailed and morphologically accurate models of mites, representing the first application combining confocal laser scanning microscopy with 3D printing to generate large physical models of mites. For this study we chose to examine six agriculturally important mites ranging in size from 110μm to 3mm, with a variety of distinct morphologies. The mites in this study include two predatory mites, *Cheyletus* sp. and *Neoseiulus cucumeris*, both of which are used as biocontrol agents of microinsects such as scales, whiteflies, thrips, aphids and other mites [1]. We also examined two mites, *Phyllocoptes fructiphilus* and *Brevipalpus* sp., which have known associations with viruses affecting roses and citrus, respectively [2, 3]. Additionally, we investigated two mites that parasitize honeybees and are major contributing factors to colony collapse disorder, *Varroa destructor* and *Tropilaelaps mercedesae* [4, 5].

A Zeiss LSM710 confocal laser scanning microscopy (CLSM) system was utilized to acquire primary three-dimensional data. Mites were mounted in glycerin between two coverslips along with spacers of equal depth as the mites. Images were captured using a Zeiss Axio Observer inverted microscope with 40x 1.2 NA Plan-Apochromat objective. Three excitation wavelengths were utilized, 405nm (DAPI), 488nm (GFP) and 561nm (DsRed) with a broad filter set capturing all emission from 410nm to 704nm wavelengths, with a pin hole of 33µm. Zeiss Zen 2012 Pro software was used to obtain 20-150 z-stack images of the dorsal and ventral side of the mites to produce CZI files which render the sample in three dimensions (Fig 1A).

CZI files are converted into OBJ files using FIJI [6], and filters in MeshLab 2016 are used to "remove isolated pieces", reducing noise and artefacts. AutoDesk Meshmixer is used to further refine the model and ensure all appendages are positioned for optimal printing (Fig 1B). Since the 3D printer prints from the bottom upward the model is typically cut in half into two pieces with the cut sides oriented downward (Fig 1C). The STL files are then imported into Ultimaker Cura 3.1 and removable support structures are added underneath delicate parts of the model where overhangs occur. Various settings are adjusted as needed to increase the thickness of the outer shell, fill in unwanted holes in the interior, and adjust the density of the honeycomb infill of the interior. The model is then sliced and converted into G-code and printed with PLA (Polylactic Acid) with an Ultimaker2 3D printer. Depending on the size and complexity of the model the 3D printing process typically takes from 24 to 72 hours to complete. Afterwards, the support structures

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are removed from the model and the two halves of the mite are glued together using cyanoacrylate glue (Fig 1D).

The production of these models has numerous benefits including the education of diverse target audiences. These models can be used as instructional tools in a classroom setting and as tools for science outreach. We are particularly interested in the possibility of using these models at ports of entry to train border inspectors to identify and intercept potentially devastating mites before they enter the country. Similar to the use of ball-and-stick models for chemical compounds, the value of being able to hold and physically manipulate a model in one's own hands cannot be overstated and may lead to enhanced scientific understandings of these mites. Our models can aid not only individuals who are naïve to mites, but also experienced researchers who are now able to directly visualize these microscopic organisms [7].

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[7] Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer. The authors acknowledge the contribution of samples from Fabio Aschaki, José Rezende, Samuel Ramsey, USDA-ARS, University of Maryland and the Smithsonian NMNH.

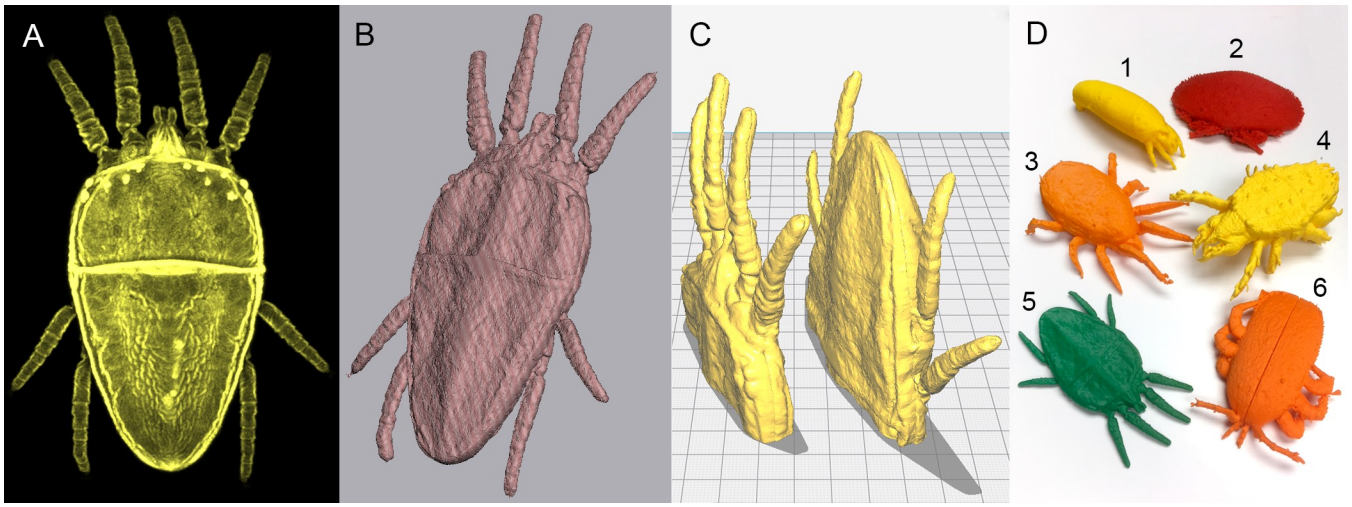

Figure 1. 3D Models of Mites. (A) Confocal microscopy image of *Brevipalpus* sp. (B) 3D model of *Brevipalpus* sp. in Meshmaker (C) 3D model of *Brevipalpus* sp. halved and oriented for 3D printing in Cura (D) 3D printed models of all 6 mites 1) *Phyllocoptes fructiphilus* 2) *Varroa destructor* 3) *Neoseiulus cucumeri* 4) *Cheyletus* sp. 5) *Brevipalpus* sp. 6) *Tropilaelaps mercedesae*

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