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Ultra-High Macro Photography of Bark Beetles

Demian F Gomez[1], Andrew J. Johnson[1]

[1]University of Florida

1 Works for me dx.doi.org/10.17504/protocols.io.bnvgme3w

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol explains the process of taking high quality photos of bark and ambrosia beetles, creating the image stacks, and processing the images in image editing software (Gimp or Photoshop).

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

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Camera: Taking photos

Our camera: we use a Canon EOS Rebel T3i attached to an Olympus stereo microscope (SZX16) with a Martin's Microscope DSLR adapter (https://www.martinmicroscope.com). We use a 1X objective with a nosepiece that allows for changeover from stereo position (stereoscopic view) to mono position (on-axis view to focus straight).

EOS Utility and turn camera on

Camera Settings - Remote shooting

Live view shoot

Folder / Where to save picture

Light diffuser: vellum or tracing paper under silver arm (or any type of white, translucent paper), glass slide for moving specimens, remouldable putty (e.g. blu-tac, white version best)for pinned specimens. White paper under specimen if putty is not white.

Settings 100 ISO, adjust shutter speed so that no pixels are over-exposed (⅛ on stereo scope, 1/30-1/100 on compound scope). Use white paper to set white balance before taking the pictures.

Check RGB graph

Disable: quick preview automatically, software link none.

Micro moves 10 to 10.

Helicon (https://www.heliconsoft.com**) for stacking photos**: for stacking use method C

Photoshop (https://www.adobe.com/products/photoshop.html**) or Gimp (**https://www.gimp.org**)**

Correct with balance first. Click options within levels. Ctrl +L

Magic wand with tolerance 9: shift is + , alt is -

Quick selection tool: select background in pieces.

Lasso tool: modify small pieces of selection. Add to selection / substract of selection.

Feather and Contract the same amount of pixels.

Right click / feather: smooth lines.

Select / Modify / Contract: expand selection.

Image / Levels: start with modify levels of white.

Delete background: selection.

Size selection: Edit > Transform > Scale, Rotate, Skew, Distort, Perspective, or Warp.

To remove dust or clean images, use the Spot Healing Brush: with large zoom, press J. This tool is used to clone areas from an image and blend the pixels from the sampled area seamlessly with the target area.

Shape Tools group, Custom Shape Tool: to add pointers, scale, or arrows.

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