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Microtome Dissection of Beetles

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Abstract

This protocol describes how visualize internal structures, from mandibular mycangia, to beetle heads or bodies.

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.

Troubleshooting

Fixating

For visualizing mandibular mycangia, beetle heads or bodies should be fixed in 96% ethanol, immersed in 30% hydrogen peroxide for 24 h, and embedded in paraffin.

Microtome

Preparates can be sectioned on a microtome at 5 µm and stained with hematoxylin (to emphasize nuclei in fungi) and eosin (to visualize proteins, muscles, and other beetle tissue).

Important points

1. The immersion in hydrogen peroxide is critical for softening beetle exoskeleton and to avoid fracturing.

The angle of beetle immersion in the wax is also critical. It depends on what mycangia you are interested in, what angle you want to slice them, and what is the angle of attachment of the wax sample to your microtome. You may need to play with the beetle position as the wax is hardening.