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Beetle Cleaning

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Bark Beetle Mycobiome Research Coordination Network Bark

Beetle

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Abstract

This protocol describes surface-sterilization or surface-cleaning techniques for the beetles before the microbe isolation.

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, Seltnann 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>.

Many researchers who study microbial associates of bark/ambrosia beetle apply various surface-sterilization or surface-cleaning techniques to the beetles before the microbe isolation, in attempt to minimize contamination. We do not know if this is important, because we have not tested whether cleaned beetles have a higher chance of success. We do not surface-wash beetles and grind them up by default. Instead, we focus on the organ in question. Mostly that is the mycangium, or the gut, or the surface itself. We advocate for a nuanced sampling of fungi from beetles, cognizant of the fact that the location of a fungus on/in the beetle body is an essential part of the ecological information.

If needed, for Surface Washing:

Ingredients:

Gloves

Handle one sample at a time

Sterilize Falcon tube

5 ml of PBS + 45 ml DI water (sink) + 50 uL Tween 80 (brown glass bottle)

Autoclave Falcon tube

Glass Petri dish

Instructions:

Drop sample in Petri dish

Add PBS to vial (with disposable pipet), fill to half the vial

Vortex 15 seconds at speed 2000

Drop sample in Petri dish

Clean Petri dish from PBS

Clean vial with ethanol

Add ethanol to vial, fill to half the vial.

Vortex 15 seconds at speed 2000

Drop sample in Petri dish

Add ethanol and store

Passive cleaning

Before introducing beetles into new tubes, pass them through several vials with moist kimwipe (all sterile), two days in each vial. This way they empty out their guts, attached spores, and mites. IMPORTANT – poke a hole in the vial lid, otherwise beetles quickly suffocate.