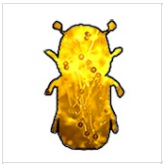

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Beetle Cleaning

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

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes surface-sterilization or surface-cleaing 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, 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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Many researchers who study microbial associates of bark/ambrosia beetle apply various surface-sterilization or surface-cleaing 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.

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