

Protocol for dissection and tissue preparation of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae) for morphometric analysis and morphological analysis using scanning electron microscopy

Alina Avanesyan¹, Timothy K. Maugel², and William O. Lamp¹

¹Department of Entomology, University of Maryland, College Park, Maryland, United States of America,

²Laboratory for Biological Ultrastructure, University of Maryland, College Park, Maryland, United States of America

Step 1. Insect collecting and preserving

1. Collect insects and immediately place them in small glass or plastic vials with 80% ethanol
2. Transport vials with insects to the laboratory and store them in the fridge at 4 °C until dissection.



Step 2. Dissection and tissue preparation

1. Place individual insect on a microscope slide.
2. Under the dissecting microscope separate the head with the mouthparts from the insect body.



3. Isolate the labium and expose the stylets using a pair of fine tweezers from the micro dissecting kit (BioQuip Products Inc., Rancho Dominguez, CA, USA; micro dissecting kit, Cat. No. 4761).



4. Similarly, separate the tarsus from one of the forelegs using the micro slide tool kit (BioQuip Products Inc., Rancho Dominguez, CA, USA; micro slide tool kit, Cat. No. 4831).



Step 3. Morphometric measurements

1. For each individual insect, photograph the head with the mouthparts, the labium, the stylet fascicle, and the dorsal view of the tarsal tip (Zeiss Axio-Imager M1 using Zeiss ZEN imaging software (Carl Zeiss, Jena, Germany)).
2. Using the photographs, take the following 12 morphometric characteristics:
 - 1) Distance from the labial tip to the base of the first labial segment; μm ;
 - 2) Distance from the labial tip to the base of the last labial segment; μm ;
 - 3) Maximum width of the last labial segment; μm ;
 - 4) Distance from the tip of the stylet fascicle to the base of the stylets; μm ;
 - 5) Distance from the apex of stylet fascicle extended from labial tip to the labial tip; μm ;
 - 6) Distance between tarsal claw tips from the dorsal view; μm ;
 - 7) Distance between bending centers of the external arcs of the tarsal claws from the dorsal view; μm ;
 - 8) Distance between the lateral margin of the arolium and tarsal claw tips from the dorsal view; μm ;
 - 9) Distance between the lateral margin of the arolium and bending centers of the external arcs of the tarsal claws from the dorsal view; μm ;
 - 10) The maximum anterior width of the arolium; μm ;
 - 11) Length of the lateral margin of the arolium; μm ;
 - 12) The angle between the lateral margins of the arolium from the dorsal view; degrees.

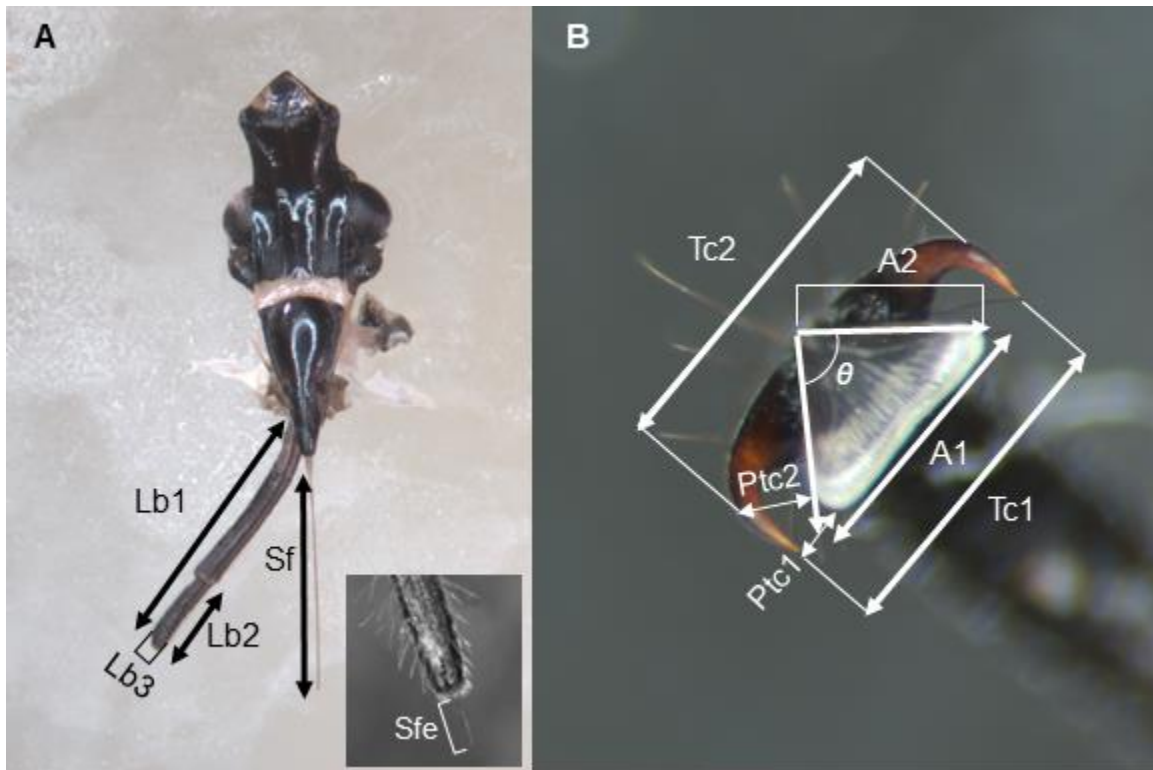


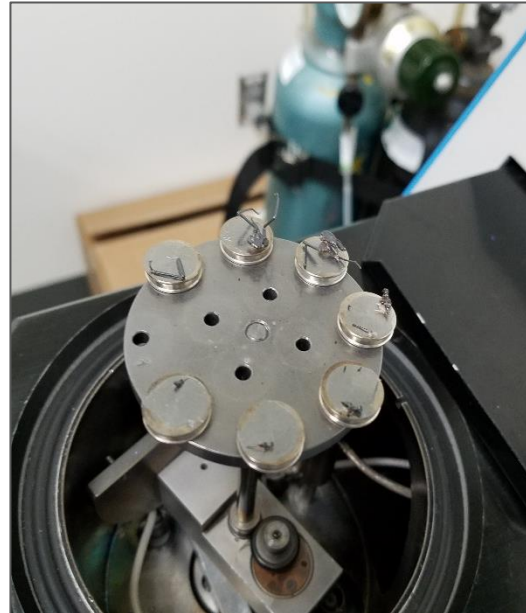
Fig 1 (from the manuscript). Morphometric characteristics measured for the labium, the stylet fascicle and the tarsal tip of *Lycorma delicatula*. (A) Labium and stylets. Lb1, distance from the labial tip to the base of the first labial segment; Lb2, distance from the labial tip to the base of the last labial segment; Lb3, maximum width of the last labial segment; Sf, distance from the tip of the stylet fascicle to the base of the stylets; Sfe, distance from the apex of stylet fascicle extended from labial tip to the labial tip. (B) Tarsal tip, the dorsal view. Tc1, distance between tarsal claw tips; Tc2, distance between bending centers of the external arcs of the tarsal claws; Ptc1, distance between the lateral margin of the arolium and tarsal claw tips; Ptc2, distance between the lateral margin of the arolium and bending centers of the external arcs of the tarsal claws; A1, the maximum anterior width of the arolium; A2, length of the lateral margin of the arolium; θ , the angle between the lateral margins of the arolium.

3. Transfer the isolated mouthparts and tarsi back to 80%-ethanol for scanning electron microscopy.

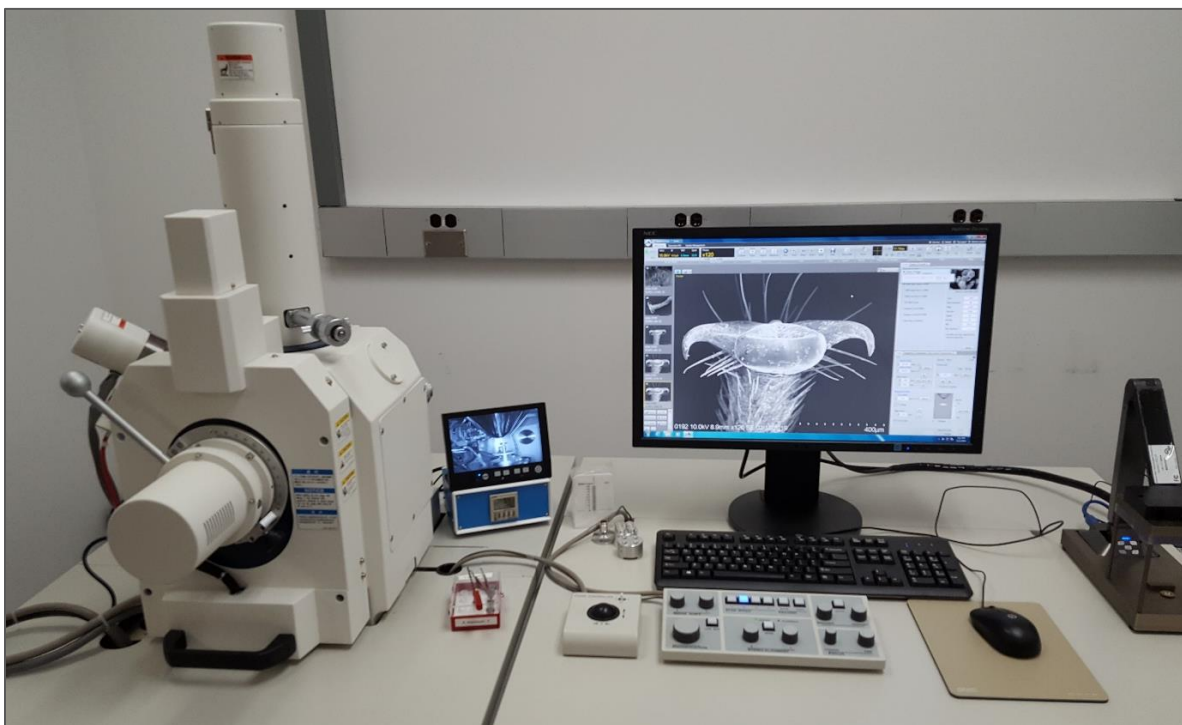
Step 4. Scanning electron microscopy and image processing

1. Dehydrate the mouthparts and tarsi by transferring them from 80% ethanol to 95%-ethanol for 10 min and then to 100%-ethanol, three times for 10 min.
2. Immerse the specimens in a graded series of 100%-ethanol and 100%-hexamethyldisilazane (HMDS), 2:1, 1:1, and 1:2 for 10 min each.
3. Immerse the specimen in 100%-HMDS for three changes of 15, 30, and 45 min.
4. After the last HMDS change, just cover the specimens by fresh 100%-HMDS, move them to a vacuum desiccator for air drying at room temperature (overnight).

5. Mount the mouthparts and tarsi to stubs and coat them with 10 nm of gold/palladium in a sputter coater.



6. Examine the specimens and image them using a scanning electron microscope (Hitachi SU-3500).



7. Take a photograph of (a) the labium and stylets, and (b) tarsal claws and arolia, for each developmental stage. Additionally, at the labial tip record the presence (or absence) of different types of labial sensilla at each developmental stage.