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Title: Clear Resin Casting of Arthropods for Use in Education, Outreach, and Research

Authors and Affiliations:
Macie Garza[1], Rachel Curtis-Robles[2], Sarah Sittenauer[1], Gabriel L. Hamer[1]

[1]Department of Entomology, Texas A&M University
[2]Department of Veterinary Integrative Biosciences, Texas A&M University

Corresponding Authors:
Gabriel L. Hamer ghamer@tamu.edu

Email Addresses for All Authors:
Macie Garza macie.garza12@gmail.com
Rachel Curtis-Robles rcurtis@cvm.tamu.edu
Sarah Sittenauer sarah.sittenauer@ag.tamu.edu
Gabriel L. Hamer ghamer@tamu.edu

Author Questionnaire

1. **Microscopy**: Does your **protocol section** require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? 1 mile away (we can have a guest parking space next to both buildings).

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length
Number of Steps: 24
Number of Shots: 44

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

1.1. **Gabriel Hamer:** Our goal is to create insect mounts that provide good visual clarity while protecting the specimen from extensive handling and to educate students and the public.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Macie Garza:** Soft bodied or fragile insects are more difficult to retain the morphological features and minimize air bubbles while embedding in resin.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

1.3. **Gabriel Hamer:** Many educational and extension activities for insects do not have commercial suppliers where specimens can be purchased. This protocol shows how others in this field can generate their own resin products.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.4. **Gabriel Hamer:** High quality specimens preserved in resin will increase professional and public recognition for different species that are pests or vectors of infectious pathogens.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.5. **Gabriel Hamer:** These specimens advance the ability to use community science for research projects and to reduce human, animal, and plant diseases vectored by insects. **NOTE**: The statement was modified by the author while shooting

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Specimen and Label Preparation

Demonstrator: Sarah Sittenauer

2.1. To begin, remove the ticks from 70 percent ethanol **[1]**. Place adult and nymphal ticks removed from ethanol between two microscope slides **[2]** and apply gentle pressure to both sides to remove excess ethanol from the body **[3]**. Transfer the tick onto a flat surface and allow it to dry for at least 5 minutes before placing it into resin **[4]**.

2.1.1. WIDE: Talent removing ticks from a storage vial labeled either "70% Ethanol".

2.1.2. Talent placing a tick between two microscope slides.

2.1.3. Talent gently pressing on either side.

2.1.4. Talent placing the tick on a table surface.

2.2. Place larvae or pupae from a mosquito colony or field-collected samples into water inside an emergence chamber **[1]**. After eclosion, place the mosquitoes in the freezer to kill them **[2]**. Then, remove the mosquitos from the freezer and leave them at room temperature for at least 5 minutes before embedding in resin **[3]**. When ready to embed in resin collect the newly emerged adult mosquitoes from the top compartment of the emergence cup **[4]**.

2.2.1. Talent placing mosquito larvae or pupae into an emergence chamber filled with water.

2.2.3, Talent placing mosquitoes into the freezer.

2.2.4, Talent removing mosquitoes from the freezer and keeping them on the work bench at room temperature.

2.2.2, Talent opening the top compartment of the emergence cup and collecting adult mosquitoes.

Author's NOTE:

[Shot order should be 2.2.1, 2.2.3, 2.2.4, then 2.2.2.] VO is rearranged accordingly

2.3. Next, remove mosquito or other Diptera larvae from ethanol **[1]** and soak them in resin catalyst for at least 20 minutes before embedding them in resin **[2]**.

2.3.1. Talent taking larvae from a vial containing ethanol.

2.3.2. Talent placing the larvae into a container filled with resin catalyst.

2.4. For other arthropods, determine whether to follow the tick or mosquito preparation steps based on the fragility of external features **[1]**.

2.4.1. Talent examining another arthropod.

2.5. Create labels with required information such as scientific names and life cycle stages **[1]**. Print the labels on transparency sheets using a laser printer, not an inkjet printer **[2]** and cut the printed labels to the appropriate size **[3]**.

2.5.1. Talent typing label information on a computer.

2.5.2. Show label text printed on transparency sheets.

2.5.3. Talent cutting the printed labels into small rectangles.

2.6. Use a size 8 font in Times New Roman italicized for general specimens and for life cycles or larger-bodied specimens, apply a size 12 font in Times New Roman italicized **[1]**.

2.6.1. Talent shows printed sheet with Size 8 and then the one with Size 12 font.

2.7. For teaching collections, incorporate 1 square centimeter QR codes on the labels that link to a website with species information when scanned with a smartphone **[1]**.

2.7.1. Show the QR code label stuck to a sample.

3. Adding the Resin Layers

3.1. Put on nitrile gloves before handling uncured resin or catalyst **[1]**.

3.1.1. WIDE: Talent wearing nitrile gloves in preparation for working with resin materials.

4.1,. Place a wooden block into the pressure pot to elevate the surface for positioning the silicone molds **[1]**.

4.1.1, Talent inserting a wooden block at the base of the pressure pot and adjusting it to lay flat. **Author's NOTE**: [4.1 should be moved after 3.1]

3.2. Measure approximately 45 milliliters of resin into a plastic cup **[1]** and pour the resin into a plastic bowl **[2]**. Place the bowl in a water bath set at 32 degrees Celsius to warm **[2]**.

3.2.1. Talent measuring and pouring 45 milliliters of resin into a plastic cup.

3.3.1,. Talent pouring resin from plastic cup into plastic bowl. **Author's NOTE**: [Please move 3.3.1 after 3.2.1. Combined shots of 3.2.1, 3.3.1, and 3.2.2.

3.2.2. Talent placing the plastic bowl into a water bath set to 32°C.

3.3. ~~Then, pour the warmed resin into a plastic bowl **[1]** and~~ Add 16 drops of catalyst, equivalent to approximately 720 microliters to the warmed resin in the plastic bowl **[2]**. Then, use a plastic spoon to slowly and carefully mix the resin and catalyst together for about 1 minute **[3]**.

3.3.1. ~~Talent pouring warmed resin from the plastic cup into a plastic bowl~~**Author's NOTE**: [Please move 3.3.1 after 3.2.1

3.3.2. Talent adding 16 drops of catalyst to the resin in the bowl.

3.3.3. Talent mixing the resin and catalyst slowly with a plastic spoon.

3.4. Now, pour the resin and catalyst mixture into a silicone mold that is placed on a wooden block inside the pressure pot **[1]**.

3.4.1. Talent pouring the mixture into a silicone mold positioned on a wooden block inside the pressure pot.

3.5. Wait for approximately 5 to 20 minutes until the resin becomes tacky and begins to harden before inserting the specimen **[1]**.

3.5.1. Shot of the surface of the resin to confirm tackiness.

3.6. Next, place the specimen at the center of the first resin layer, ensuring that diagnostic features such as spiracles or appendages are properly oriented for clear visibility **[1]**. Then, position the printed label on the first resin layer close to the specimen as desired **[2]**.

3.6.1. Talent using tweezers to carefully position the specimen in the center of the resin layer.

3.6.2. Talent placing the trimmed transparency label near the specimen on the resin layer.

3.7. Prepare a second resin-catalyst mix as demonstrated earlier **[1]** and slowly pour it over the specimen along the inner edges of the mold, filling the remaining space **[2]**.

3.7.1. Talent picking up the second resin cup.

3.7.2. Talent slowly pouring the second resin-catalyst mix over the embedded specimen and around the mold edges.

3.8. Adjust the position of the specimen's appendages such as legs, wings, or antennae as needed before the resin fully cures **[1]**.

3.8.1. Talent using fine-tipped tweezers to reposition the specimen's appendages carefully within the resin.

4. Pressure Pot Processing and Final Product Finishing

Demonstrators: Sarah Sittenauer, Macie Garza, Elise Hoffman, Allison Speed

4.1. ~~Place a wooden block into the pressure pot to elevate the surface for positioning the silicone molds **[1].**~~

4.1.1. ~~Talent inserting a wooden block at the base of the pressure pot and adjusting it to lay flat.~~ **Author's NOTE**: [Shot moved to after 3.1]

4.2. Close the pressure pot lid securely **[1].** Apply pressure until the gauge reads 60 pounds per square inch and incubate for approximately 24 hours **[2]**.

4.2.1. Talent closing and locking the lid of the pressure pot.

4.2.2. Talent adjusting the pressure dial.

4.3. After incubation, release the pressure from the pot **[1]** and carefully remove the molds **[2]**.

4.3.1. Talent turning the release valve.

4.3.2. Talent lifting out the molds from the pressure pot.

4.4. Take the hardened resin blocks out of the silicone molds and allow them to rest at room temperature until further processing **[1]**.

4.4.1. Talent taking out the resin blocks and setting them on a work surface.

4.5. Using a bandsaw, cut the resin blocks to the desired dimensions **[1]** and sand all six sides of each resin block using a disc sander **[1]**.

4.5.1. Talent operating a bandsaw to trim the resin block edges evenly.

4.5.2. Talent sanding each side of the resin block using a disc sander.

4.6. Repeat sanding on all sides with a 4 by 30-inch 600 grit belt sander, then with a 1 by 30-inch 2000 grit belt sander **[1]**.

4.6.1. Talent sanding the resin block again with a 4 x 30 inch 600 grit belt sander.

4.7. Next, gently press a red aluminum oxide buffing polish stick against the spinning buffing wheel for a few seconds **[1]**. Then, hold the resin block against the buffing wheel at a 45-degree angle to begin polishing **[2]**.

4.7.1. Talent applying red aluminum oxide polish to the buffing wheel.

4.7.2. Talent polishing the resin block by holding it at an angle against the spinning buffing wheel.

4.8. Use a cloth or fabric to wipe off excess polish from all sides of the resin block **[1-TXT]**.

4.8.1. Talent wiping the resin block with a cloth to remove polish residue. **TXT: Repeat this step as needed to obtain a clear surface**

4.9. Repeat the polishing steps using a second buffing wheel and a white diamond polish **[1]** and continue polishing until the colored overcast from the red polish is completely removed **[2]**.

4.9.1. Talent applying white diamond polish to a second buffing wheel.

4.9.2. Shot of the resin block appearing optically clear and free of red tint.

Results

5. Results

5.1. Successfully embedded specimens were surrounded by clear and colorless resin, with all sides smooth and the specimen clearly visible from any angle of the resin block **[1]**.

5.1.1. LAB MEDIA: Triatoma gerstaekeri (dorsal) [5.1.1].mp4

5.2. Features important for identification, such as color patterns, scale shape and size, and other anatomical details, were visible **[1]**.

5.2.1. LAB MEDIA: Culex tarsalis [5.2.1].mp4

5.2.2. LAB MEDIA: Ixodes scapularis -2 [5.2.2].mp4.

5.2.3. LAB MEDIA: Amblyomma americanum [5.2.3].mp4.

5.2.4. LAB MEDIA: Ornithodoros turicata [5.2.4].mp4.

- Ethanol

Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol
IPA: /ˈɛθəˌnɔːl/
Phonetic Spelling: ETH-uh-nawl

- Nymphal

Pronunciation link: https://www.merriam-webster.com/dictionary/nymphal
IPA: /ˈnɪmfəl/
Phonetic Spelling: NIM-fuhl

- Larvae

Pronunciation link: https://www.merriam-webster.com/dictionary/larvae
IPA: /ˈlɑːrˌviː/, /ˈlɑːrˌvaɪ/
Phonetic Spelling: LAHR-vee, LAHR-vye

- Pupae

Pronunciation link: https://www.merriam-webster.com/dictionary/pupae
IPA: /ˈpjuːˌpiː/
Phonetic Spelling: PYOO-pee

- Eclosion
Pronunciation link: https://www.merriam-webster.com/dictionary/eclosion
IPA: /ɪˈkloʊʒən/
Phonetic Spelling: ih-KLOH-zhuhn

- Diptera
Pronunciation link: https://www.merriam-webster.com/dictionary/Diptera
IPA: /dɪˈptɛrə/
Phonetic Spelling: dip-TEHR-uh

- Arthropods
Pronunciation link: https://www.merriam-webster.com/dictionary/arthropod
IPA: /ˈɑːrθrəˌpɑːd/
Phonetic Spelling: AHR-thruh-pod

- Nitrile
Pronunciation link: https://www.merriam-webster.com/dictionary/nitrile
IPA: /ˈnaɪˌtraɪl/
Phonetic Spelling: NYE-tryl

- Catalyst
Pronunciation link: https://www.merriam-webster.com/dictionary/catalyst
IPA: /ˈkædəˌlɪst/
Phonetic Spelling: KAT-uh-list

- Microliters
Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
IPA: /ˈmaɪkroʊˌliːtər/
Phonetic Spelling: MY-kroh-lee-ter

- Celsius
Pronunciation link: https://www.merriam-webster.com/dictionary/Celsius
IPA: /ˈsɛlsiəs/
Phonetic Spelling: SEL-see-uhs

- Spiracles
Pronunciation link: https://www.merriam-webster.com/dictionary/spiracle
IPA: /ˈspɪrəkəl/
Phonetic Spelling: SPIR-uh-kuhl

- Appendages
Pronunciation link: https://www.merriam-webster.com/dictionary/appendage
IPA: /əˈpɛndɪdʒ/
Phonetic Spelling: uh-PEN-dij

- Silicone
Pronunciation link: https://www.merriam-webster.com/dictionary/silicone
IPA: /ˈsɪlɪˌkoʊn/
Phonetic Spelling: SIL-ih-kohn

- Incubate
Pronunciation link: https://www.merriam-webster.com/dictionary/incubate
IPA: /ˈɪŋkjəˌbeɪt/
Phonetic Spelling: ING-kyuh-bayt

- Bandsaw
Pronunciation link: https://www.merriam-webster.com/dictionary/bandsaw
IPA: /ˈbændˌsɔː/
Phonetic Spelling: BAND-saw

- Aluminum
Pronunciation link: https://www.merriam-webster.com/dictionary/aluminum
IPA: /əˈluːmɪnəm/
Phonetic Spelling: uh-LOO-muh-nuhm

- Oxide
Pronunciation link: https://www.merriam-webster.com/dictionary/oxide
IPA: /ˈɑːksaɪd/
Phonetic Spelling: OCK-syde

- Optically
Pronunciation link: https://www.merriam-webster.com/dictionary/optically
IPA: /ˈɑːptɪkli/
Phonetic Spelling: OP-tik-lee

- Triatoma gerstaeckeri
Pronunciation link: No confirmed link found
IPA: /ˌtraɪəˈtoʊmə ˌgɝːˈstɛkəraɪ/
Phonetic Spelling: try-uh-TOH-muh ger-STEK-uh-rye

- Culex tarsalis
Pronunciation link: No confirmed link found
IPA: /ˈkjuːlɛks tɑːrˈseɪlɪs/
Phonetic Spelling: KYOO-leks tar-SAY-liss

- Ixodes scapularis
Pronunciation link: No confirmed link found
IPA: /ɪkˈsoʊdiːz ˌskæpjəˈlɛrɪs/
Phonetic Spelling: ik-SOH-deez skap-yuh-LAIR-iss

- Amblyomma americanum

Pronunciation link: No confirmed link found
IPA: /ˌæmbliˈɑːmə əˌmɛrɪˈkɑːnəm/
Phonetic Spelling: AM-blee-AH-muh uh-MER-ih-KAH-nuhm

- Ornithodoros turicata

Pronunciation link: No confirmed link found
IPA: /ˌɔːrnɪθəˈdɔːrɑːs ˌtʊrɪˈkɑːtə/
Phonetic Spelling: or-NITH-uh-DOR-oss too-rih-KAH-tuh