

Clear Resin Casting of Arthropods of Medical Importance for Use in Educational and Outreach Activities

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Subject Editor: Phyllis Weintraub

Received 14 January 2018; Editorial decision 2 March 2018

Abstract

Arthropod-related morbidity and mortality represent a major threat to human and animal health. An important component of reducing vector-borne diseases and injuries is training the next generation of medical entomologists and educating the public in proper identification of arthropods of medical importance. One challenge of student training and public outreach is achieving a safe mounting technique that allows observation of morphological characteristics, while minimizing damage to specimens that are often difficult to replace. Although resin-embedded specimens are available from commercial retailers, there is a need for a published protocol that allows entomologists to economically create high-quality resin-embedded arthropods for use in teaching and outreach activities. We developed a detailed protocol using readily obtained equipment and supplies for creating resin-embedded arthropods of many species for use in teaching and outreach activities.

Key words: education, identification, medical entomology, outreach, resin-mounting

The morbidity and mortality associated with arthropods or the agents of disease they transmit are a major health threat to humans and animals worldwide. A perpetual necessity for effective public health management is having quality teaching specimens of diverse hematophagous arthropods in order to train students entering the workforce on arthropod identification. In addition, outreach education on basic morphological identification is an important part of integrative pest management, especially when arthropod vectors live in and around human homes. The most common tools used in arthropod vector student training and outreach education are photographs, illustrations, or specimens that are in ethanol or mounted with pins or points (Rutz and Waldron 2010). However, photographs and illustrations do not generally provide a complete understanding of size or relative locations of three-dimensional features; and specimens in ethanol or those pinned or pointed inevitably become damaged while handling. The embedding of specimens in optically clear resin is an approach that retains the ability to see diagnostic features, while maximizing the resilience of the specimen and minimizing the risk of being exposed to infectious agents. Many commercial vendors sell arthropods embedded in resin but rarely are these products available for hematophagous species. Furthermore, most entomology training and outreach programs need to use species of local relevance. Our objective was to develop a resin-embedding protocol

that maximized the quality of the product while minimizing cost per unit. From 2013 to 2017, we experimented with multiple resin types, different molds, and different sanding and polishing steps; the following protocol is the culmination of that work. During those trials, we performed this resin process on a variety of arthropod specimens with a focus on triatomines (Hemiptera: Reduviidae), ticks (Acari: Ixodidae: Argasidae), mosquitoes (Diptera: Culicidae), scorpions (Arachnida: Scorpiones), spiders (Arachnida: Araneae), fleas (Siphonaptera: Pulicidae), and true fly adults and larvae (Diptera: Brachycera).

While producing insects mounted in resin, we received dozens of requests for these specimens from public health professionals, medical professionals, continuing education instructors, undergraduate and graduate level course instructors, K-12 educators, and fellow researchers. All requesters expressed their interest in being able to educate students and the public with a safe-to-handle visual example of important arthropods. We have also used such specimens in our own teaching and citizen science educational outreach efforts (Curtis-Robles et al. 2015), and they are well received. Given the popularity of the resin-mounted displays, and the frequency of requests, we hope that sharing the protocol we present here will provide the basis for others to also develop resin-embedded specimens useful for their own educational

efforts. The protocol will focus on the procedures used for triatomines, and we will also discuss the use of this protocol for other arthropods.

Protocol

We experimented with various resin-mounting approaches since 2013 before developing the protocol we present here. Initially, we experimented with a polyester-casting resin (Castin' Craft Clear Polyester-Casting Resin and catalyst, Environmental Technology, Inc., Fields Landing, CA) and polypropylene-casting molds. Specimens were allowed to dry overnight before the casting process began. Casting was completed in 3–4 layers, with a base layer that was allowed to partially harden, then the specimen was placed and a partial layer was poured to keep the specimen in place. After the placement layer was partially hardened, an additional 1–2 layers were poured to cover the specimen. The final pour and cast was allowed to cure for at least 24 h. We quickly realized that the polypropylene molds were impossible to release the cast specimen from the mold, even when using a manufacturer-suggested release product (Castin' Craft Mold Release & Conditioner, Environmental Technology, Inc.). We then tried using silicon baking molds to create small (approximately $3.8 \times 3.8 \times 2$ cm) molds of specimens such as ticks, and larger (approximately $5 \times 7.6 \times 3$ cm) molds of specimens such as triatomines. The silicon molds allowed for easy release of the cast specimens after hardening. The less-than-optimal characteristic of products cast in silicon molds was that the surfaces and edges of the resin were not uniformly flat/square, since the silicon molds were somewhat flexible and had not maintained the exact same positioning during the pouring/curing process. These curved edges distort the anatomy of the mounted specimen while looking through a microscope, which compromise the educational value. Additional problems seemed to stem from the particular resin we were using: 1) there were quite a few bubbles in the resin as it was prepared and poured, and although many of the bubble dissipated by the time the cure was set, there were cases where bubbles remained trapped in the resin, which did not create the kind of high-quality specimen we were aiming for as a final product; 2) despite trying many different ratios of resin: catalyst and many different curing situations (multiple days, different temperatures, different humidity), we were unable to ever produce a product that was not tacky to the touch (we were not able to use the manufacturer-suggested 'surface curing agent' to avoid the tacky surface because it would have created a cloudy, rather than clear, final resin). We were able to compensate slightly by applying a layer of clear liquid nail polish as an outer layer; however, after a few months of regular handling, the polish layer began to appear smudged and needed to be re-applied. An additional challenge of using presized molds was that, when placing the insects in the resin, sometimes the specimen did not stay exactly centered in the resin; eventually, we found that using a nonpermanent mold (such as a Petri dish) allowed us to cut/trim the final product to the exact size we wished. Although all these attempted methods and developments resulted in a product acceptable for occasional and short-term use for education, we pursued methods to eliminate issues and create a product suitable for heavy long-term use.

Here, we describe the most successful method that results in the clearest, most professional-appearing product. This preparation is enough to make 5 to 6 resin-encased triatomine insects.

Specimen Preparation

Specimens are typically stored in 70% to 80% ethanol (ideal for retain pliability for positioning) and air dried for 5–30 min before casting in resin. It is important for the specimen to be dry at time

of casting. We have had success with specimens coming from many different storage histories; specimens that have been refrigerated or frozen should be allowed to completely dry before processing.

First Layer of Resin

In a chemical fume hood or other well-ventilated area, mix vigorously for 1 min: 4 oz of TAP Clear-Lite Casting Resin (TAP Plastics, San Leandro, CA) with 32 drops of MEKP Methyl Ethyl Ketone Peroxide Liquid Catalyst (TAP Plastics). Carefully pour into the mold container. In this case, the resin manufacturer suggests using a rigid mold (rather than a flexible rubber or silicon mold). We used 150×15 mm Petri dishes (Corning Falcon Bacteriological Petri Dishes with Lid, Corning 351058, Corning, NY).

Adding Specimens

Continually check resin for thickness/consistency, using an object such as forceps or a small wooden stick. Reaching the proper consistency can take 20–50 min, depending on specific mixed amounts, type of container used, and other extrinsic factors such as room temperature and humidity. Using the resin amounts and Petri dish listed above, and at a temperature of approximately 23°C and humidity of approximately 51%, we found the time to reach this consistency was approximately 25–35 min. When resin begins to thicken, but before it reaches a gelatinous state, set specimens in resin and push down slightly so that the specimen slightly floats in the resin (Fig. 1A).

Second Layer of Resin

Once the resin has reached a gelatinous state, approximately 5 min after adding the specimens, add a second layer of resin (freshly mixed 4 oz of resin with 32 drops of catalyst) on top, this will allow the specimens to cure correctly without floating into other specimens or areas that cannot be properly cut out. Let the resin cure overnight (12–20 h) at room temperature in the fume hood or other well-ventilated area.

Cutting

Depending upon the sizes and weights of the specimens, they may have slightly moved during the setting and pouring of the second layer of resin. Cutting the resin allows flexibility in dealing with specimens that have floated off-center. Once the resin has completely cured, remove resin-encapsulated specimens from the Petri dish, and cut to preferred size and shape (Fig. 1B). Proper personal protective equipment should be used, including safety glasses and appropriate respirator protection. We used a benchtop bandsaw to cut specimens into desired square or rectangular blocks (Grizzly G0803-9" Benchtop Bandsaw, Grizzly Industrial, Inc., Springfield, MO).

Sanding

The sanding process begins with the sanding the imperfections from the outer surfaces of the resin blocks. We used a disc and belt sander (Grizzly H6070 $1' \times 30'$ Belt/5" Disc Sander, Grizzly Industrial, Inc.) to sand the resin (Fig. 1C). We first used a disc sander with medium grit sand paper (P80/P180) (Norton 5"-80 grit R228 Aluminum Oxide PSA Disc, Norton 5"-180 grit R228 Aluminum Oxide PSA Disc, Saint-Gobain Abrasives Inc., Worcester, MA) to smooth all the sides of the resin. Then we used a belt sander with increasingly fine grit sand paper (P500, P1200, P3000) (Klingspor 1x30-500 CS321X Silicon Carbide, KLINGSPOR Abrasives, Inc., Hickory, NC; Norton 1x30-1200 X16 U254 Norax Aluminum Oxide Engineered Abrasive, Norton 1x30-3000 X5 U254 Norax Aluminum Oxide Engineered Abrasive Saint-Gobain Abrasives Inc.) to completely smooth all the sides of the resin. The final sanding with P3000 sand paper ensures

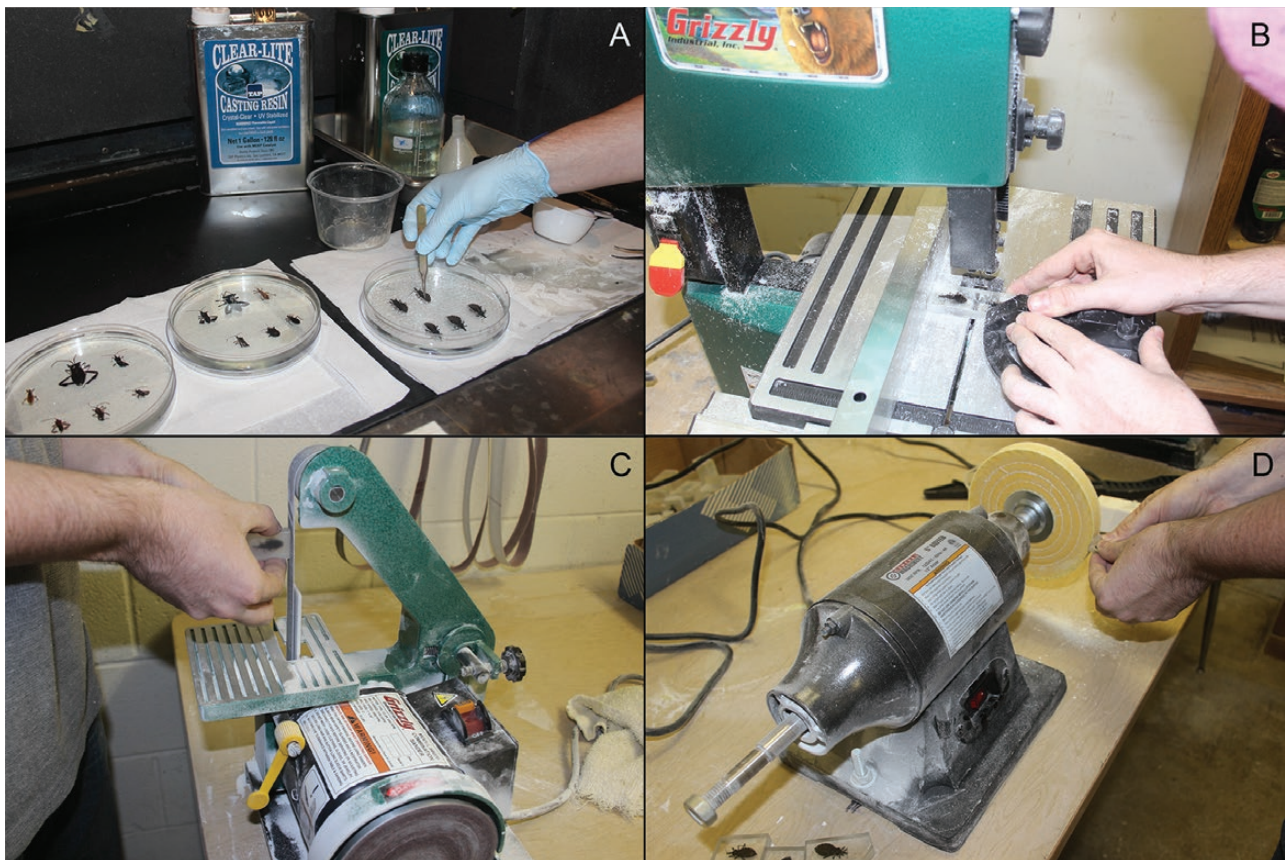


Fig. 1. Clear-casting resin production for arthropods showing resin setting in fume hood (A), cutting resin into blocks using band saw (B), sanding resin blocks using belt sander (C), and polishing resin using buffing wheel (D).

efficiency during the buffing/polishing phase. Creating completely smooth surfaces is particularly important for specimens that may be viewed under a dissecting scope, since flat surfaces allow for better viewing with less light and objective distortions.

Buffing and Polishing

We used a buffer (Central Machinery 6" Buffer, 1.6 amp, 3,450 RPM, Camarillo, CA) with white compound buffer sticks (Woodstock, 1 lb White Buffing Compound D2903, Woodstock International, Inc., Bellingham, WA) and buffing wheel (Extra Thick Spiral Sewn Buffing Wheel, 6 inch, 80 Bly, Enkay Products Corp., Edgewood, NY) (Fig. 1D). Each edge was applied to the buffing wheel and when transitioning to a new side, all compound residues were wiped off resin using a cloth or paper towel before starting a new side. Continue the buffing process until desired results of a clear appearance and flat surfaces are achieved; this will allow for morphological features to be observed under a microscope viewing.

Results and Discussion

This protocol of resin-encapsulation results in professional-looking products (Fig. 2A). Using optically clear resin and carefully buffing all sides results in a safe-to-handle specimen that can be viewed from six angles, including under a dissecting scope (Fig. 2B).

Using a commercially available clear resin-casting process, we have created a straightforward protocol for producing high-quality resin-embedded arthropods for teaching and outreach purposes. For our education and outreach activities at Texas A&M University, we have utilized resin-embedded specimens produced with this protocol

for triatomines, ticks, scorpions, spiders, fleas, and true fly adults and larvae. For the specimens that yielded best results, we have not noticed a change in their appearance over a year of storage. With excessive use of these resin blocks during teaching, the surfaces can become scratched but rebuffing these can improve transparency. To acquire the equipment and supplies necessary to this process, the cost is around \$560 (Table 1).

We now regularly use these specimens in the courses we teach at Texas A&M University Entomology Department. Between our Department's Veterinary and Medical Entomology courses, both of which have lab sections, we teach about 600 students each year. The traditional specimen mounting methods of pinning, pointing, or in ethanol was not sustainable given the persistent need to replace damaged specimens. In addition to use with students, Texas A&M AgriLife Extension entomologists and agents use the in their outreach activities and programs.

In addition to teaching activities, we have engaged in public education outreach regarding triatomine insects—vectors of *Trypanosoma cruzi*, etiologic agent of Chagas disease—for the past several years, parallel to our efforts of establishing a research collection through citizen scientists (Curtis-Robles et al. 2015). While conducting outreach, we found it useful to have examples of triatomines for the public to view, as have others (Paredes-Gonzalez et al. 2015). Others have also mentioned the importance of using resin-embedded bed bugs for outreach education (Rutz and Waldron 2010).

Another benefit of using this resin-embedding process is that it offers a secure way to preserve field-collected vectors of unknown infection statuses. For the preparer, there is no need to puncture the specimen with a pin and risk pathogen exposure; for the viewer, there

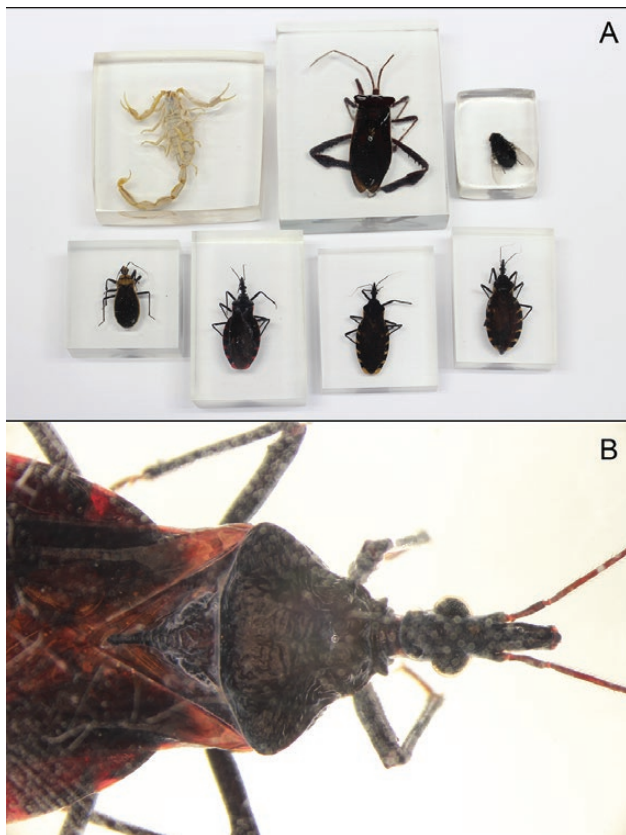


Fig. 2. Finished resin blocks containing an assortment of different arthropods (A) and an image of *Triatoma gerstaeckeri* (Stål) (Hemiptera: Reduviidae) in resin through a dissecting microscope (B).

is no risk of a specimen being mishandled and potential pathogen exposure occurring. This is especially important when infectious agents transmitted by arthropods have high resilience to inactivation such as African swine fever virus (see review in Costard et al. 2013), which may be transmitted by soft ticks (*Ornithodoros* spp (Koch) (Ixodida: Argasidae)), and *Yersinia pestis*, causative agent of plague, which may be spread by fleas (Rose et al. 2003).

While we have had success with this procedure for many specimens, there are some limitations. With the outlined protocol, we have learned that adult ticks, scorpions, fleas, and true fly adults and larvae can be substituted for triatomines with similar results. The spiders (*Latrodectus* sp. (Walckenaer) (Araneae: Theridiidae) and *Loxosceles* sp. (Heineken & Lowe) (Araneae: Sicariidae)) did not yield a good result—air gaps formed around the specimen and resulted in a distorted view. We also learned that smaller and fragile arthropods such as mosquitoes do not retain a good posture or lose key features such as scales for species differentiation while being flooded with resin. For mosquitoes, we still find it better to point-mount specimens or place in hand sanitizer in a cuvette. In addition to problems with specific groups of arthropods, resin embedding may also occasionally result in a restricted ability to clearly see minute morphological details. Particularly because of the two-layer-pouring technique (which allows us to carefully place the specimen in the center of the resin block), the distinction between the layers may be seen from the side views

Table 1. Cost summary for primary equipment and supplies needed to replicate clear resin-casting protocol outlined here

Product	Cost
Resin purchased from TAP Plastics online Clear-Lite Casting, 1-gallon	\$84.25
MEKP Liquid Catalyst for Clear-Lite Casting Resin	\$4.15
Bandsaw purchased from Grizzly online	\$199.95
Bandsaw blade replacement	\$17.95
Sander purchased from Amazon	\$150.00
Buffer purchased from Amazon	\$44.99
Sanding disc purchased from Tru-Grit online 5"-discs 80 grit	\$0.72
Sanding disc purchased from Tru-Grit online 5"-discs 180 grit	\$0.65
Sanding wheels purchased by Tru-Grit online 1x30-500	\$2.40
Sanding wheels purchased by Tru-Grit online 1x30-1200	\$3.50
Sanding wheels purchased by Tru-Grit online 1x30-3000	\$3.45
Buffing compound wheel kit purchased from Amazon	\$25.72
Buffing wheels purchased from Amazon	\$10.00
Buffing compound purchased from Amazon	\$10.90
Total amount spent:	\$558.63

Cost of shipping not included.

of the resin, which may occasionally interfere with seeing some morphological characteristics from the side view. During this process, we also experienced adverse effects of color hue when using other buffing compounds with the exception of white. The white compound produced the clearest hue without having brown or red visible hues. Despite these limitations, we have found resin-preserved arthropods to be quite useful for the majority of teaching and outreach activities.

Acknowledgments

We thank the Texas EcoLab Program and the Texas A&M University Department of Entomology for funding. This project was partially supported by the Cooperative Agreement Number, 1U2GGH001640-01, funded by the Centers for Disease Control and Prevention. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

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