DIFFERENTIATION AMONG THE NORTH AMERICAN TRIATOMINAE SPECIES (VECTORS OF THE CHAGAS DISEASE PARASITE) AND THEIR COMMONLY MISIDENTIFIED DOPPELGÄNGERS

A Thesis

by

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ABSTRACT

Chagas disease is increasingly recognized as a major public health concern in the United States. The disease is caused by infection with the protozoan parasite *Trypanosoma cruzi*, which is spread by blood-sucking insects commonly referred to as kissing bugs (Reduviidae: Triatominae). Limited outreach and educational resources are available regarding Chagas disease for the public and medical or veterinary practitioners that may encounter infected patients. A key challenge, especially in outreach and public health awareness, is differentiating the kissing bug vectors from common look-alike insects that do not feed on blood and do not pose a risk of *T. cruzi* transmission. The presence of these look-alikes, or Doppelgängers, is associated with both psychological and economic consequences, as they cause needless worry among the public and encounters with these insects have led to unwarranted human and canine blood testing for Chagas disease. In my thesis, I developed outreach materials suitable for use by the lay public as well as veterinarians, medical doctors, pest control operators, public health officials, and others to facilitate the identification of kissing bugs. First, I created identification and pictorial guides to North American Triatominae species and their common look-alikes, including a step-by-step dichotomous key to differentiate key anatomical features useful in discriminating species. Next, I developed a process for manufacturing resin-embedded kissing bugs and look-alike species resulting in high quality products that are safe to handle; these specimens will be long-lasting and valuable in outreach programs to show differences in size, shape, and color that photos alone cannot detail.

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CHAPTER I

INTRODUCTION

American trypanosomiasis, or Chagas disease, was first described in 1909 in Brazil by clinical biologist Carlos Chagas (Lent et al., 1979). Chagas disease is vector-borne disease with the insect vectors belonging to the subfamily Triatominae, which are known as kissing bugs, conenose bugs, or chinches. The etiological agent of Chagas is the protozoal parasite *Trypanosoma cruzi*. The parasite is transmitted through the feces of the vector, which is unlike most other vector-borne pathogens that are spread via arthropod bites. At least 6 million people are infected with *T. cruzi* worldwide, with more than 70 million individuals at risk (WHO-WER). Chagas disease has an annual human mortality of approximately 10,000 to 12,000 a year worldwide (WHO, 2017; DSHS Chagas Update, 2016). Chagas disease has three stages that have been described from human hosts, and it is likely that animal disease may present similarly. The incubation period of *T. cruzi* is dependent on how the parasite is acquired; oral 3-22 days, vectorial transmission 4-15 days, and blood transfusion 30-40 days (Nobrega et al., 2009). Once symptoms arise, during the acute phase of the infection, clinical presentation consists of flu-like symptoms, general abdominal pain, dyspepsia, epigastric pain, facial edema, myalgia, arthralgia, tachycardia, lymphadenopathy, splenomegaly, hepatomegaly, and/or inflammation and/or rash at infection site (Nobrega et al, 2009; WHO, 2017; PAHO/WHO, 2017; CDC, 2014). Demonstration of a symptomatic clinical presentation of the acute phase of infection occurs in approximately 20-30% of infected humans (Bern et al., 2011). The second stage, known as the latent or indeterminate stage,

is not well understood due to fluctuations in parasitemia and fewer clinical symptoms present. If a person during this stage progress with clinical symptoms such as cardiac manifestations, the individual will be considered to be in the third stage. Approximately 30% of people infected with the parasite progress to the third stage known as the chronic stage, which is characterized primarily by cardiac manifestations (Rassi et al., 2010). In humans, most clinical presentations relate to cardiac complications, while other less common forms such as gastrointestinal (esophagus or colon manifestations) and meningoencephalopathy occur (Bern et al., 2011, Rassi et al., 2010).

Chagas disease is considered a neglected tropical disease (NTD). The NTDs are a group of debilitating, poverty-promoting disease that disproportionately impact people living in poverty (Hotez et al., 2016). The NTDs receive far less funding and attention than other infectious disease that circulate infect some of the same populations, such as HIV, AIDS, and Malaria. The effects of Chagas disease morbidity have been quantified as a lifetime average of 3.57 disability-adjusted life-years (DALYs), and incurring an average lifetime cost of $27,684 for each infected individual (Lee et al., 2013). This illustrates that Chagas disease is similar via DALYs and financial burdens in relation to diseases such as Lyme disease, methicillin resistant *Staphylococcus aureus* (MRSA), helminthes and nematode infections, and many other diseases, which have more media and financial assets (Lee et al., 2013; Hotez et al., 2014).

The Chagas pathosystem has gained serious media and public attention within the United States in recent decades, due to autochthonous cases being diagnosed and recognized. Recent estimations suggest that there are approximately 238,091 people infected with *T. cruzi* within the United States, not including undocumented immigrants

that could account for an additional 88,000 to 109,000 (Montgomery et al., 2014; (Manne-Goehler et al., 2016). Within the United States, blood screening began in 2006 and has been using increasingly higher specificity tests since 2010 (FDA, 2015) (Montgomery et al., 2014). From 2007 to 2017, the American Association of Blood Banks have found 2,290 blood donors testing positive for *T. cruzi* during blood screening tests (http://www.aabb.org/research/hemovigilance/Pages/chagas.aspx). Chagas disease was recently mandated to be a reportable disease in Texas, and the Texas Department of State Health Services compiled reports of 20 autochthonous cases out of 91 total human cases from 2013 to 2016 (https://www.dshs.texas.gov/idcu/disease/chagas/data/). The rest of the cases were either imported or unknown source of infection.

Within the United States there are eleven Triatominae species; *Paratriatoma hirsuta (AZ, CA, CO, NV, NM), Triatoma gerstaeckeri (NM, TX), T. incrassata (AZ), T. indictiva (AZ, NM, TX), T. lecticularia (Southern US), T. neotomae (AZ, CA, NM, TX), T. protracta (Southwestern US), T. recurva (AZ, CA, CO, NV, NM, TX), T. rubida (AZ, CA,CO, NV, NM, TX), T. rubrofasciata (FL, HI), and T. sanguisuga (Southern US)*. Of the species, all but two have been naturally infected with *T. cruzi*, but *P. hirsuta* has been experimentally infected with *T. cruzi* presenting the possibility of being naturally infected. The most common states with infected triatomine species within the southern United States are Arizona, California, New Mexico, and Texas. Multiple studies in Texas have shown *T. cruzi* infection prevalence in triatomines at 40% to 80%, depending on species and ecoregion (Curtis-Robles, 2015; Kjos, 2013). Within Texas, there are seven species triatomine species, all of which are capable of transmitting *T. cruzi*. The most

commonly reported are *Triatoma gerstaeckeri*, *T. lecticularia*, and *T. sanguisuga*, with *T. gerstaeckeri* being the most commonly infected species (Kjos et al., 2009).

Triatomines are known as kissing bugs, conenose bugs, or chinches and present a cryptic nocturnal activity during their life stages. Both male and female bugs feed on blood to develop through their life stages into adulthood. Kissing bugs have five nymphal instar stages before becoming reproductive adults, with the first nymphal stage feeding only once while the rest may take multiple blood meals (Lent et al., 1979). All stages can transmit *T. cruzi* to a host, but *T. cruzi* infection in early instars is less common due to fewer blood meals consumed and therefore fewer chances to become infected by the host. Triatomines are attracted by different extrinsic cues similar to that of other hematophagous insects such as carbon dioxide/monoxide, heat, light (blacklight, mercury vapor, other external light sources), and movement (Lent et al., 1979). As for habitats during the day, triatomines primarily hide within animal nests, cracks, and crevices. While in nymphal stages, triatomines spend less time dispersing primarily due to their lack of wings and will associate with animals within their nests or structures, which provide regular blood meals for them. Many different hosts can be reservoirs for *T. cruzi* most of which are small to medium-sized mammals such as rodents, raccoons, armadillos, opossums, and even some larger mammals such as canines and felines (Lent et al., 1979; Bern et al., 2011).

Chagas disease is difficult to diagnose with minimal clinical manifestations being present, especially when *T. cruzi* is acquired orally, congenitally, or through blood transfusion or organ transplantation. Chagas disease can be a debilitating disease for animals and humans alike. In the absence of a human or animal vaccination and with

limited treatment options, the best way to prevent the disease is through a focus on vector control and education of the public and professionals. With concern mounting over Chagas transmission, details of the disease, and awareness of what the vectors look like is greatly needed.

Programs providing outreach-based methods for vector-borne diseases can provide great insight into the diseases as well as provide ways to prevent disease infections. Citizen science programs can aid in data generation by providing this information as well as providing a means of education to the public and health care professionals alike (Curtis-Robles, 2015). The data generated can aid in the distribution of vectors, infection status of vectors and hosts, and provide a future prospective of disease transmission. Citizen science programs benefit in the aspect of identifying kissing bugs from non-kissing bugs as well as determining disease burden of the infected bugs throughout different ecoregions where populations occur. We have developed a phone application as well as an online version for bug submission (http://kissingbug.tamu.edu). This program benefits the public because they can be easily informed and aware of kissing bugs and Chagas occurrence.

1.1 Kissing Bug Vector-Biology

1.1.1 Distribution

Triatomines are found throughout the Americas and sparse areas of Asia (Paleoarctic, Oriental regions), Africa (Ethiopian region), Australia (Australian region). Greatest species diversity is found throughout the Americas, 152 species of triatomines are known to occur (Da Rosa et al., 2017; Galvao C., 2014; Mendonca et al., 2016;

Poinar G., 2013; Souza et al., 2016). Relatively few, only 13 species, occur throughout Asia (Lent et al., 1979).

In the US, triatomines have been reported in 30 states, with more frequent reports in the southern states. Although triatomines can be found across the southern US, the most and largest studies have been completed in the southwestern US, namely Arizona, California, New Mexico, and Texas with 6 to 9 species occurring.

1.1.2 Taxonomy

Kissing bugs or conenose bugs are hematophagous "blood-feeding" insects in the Order; Hemiptera, Suborder; Heteroptera, Family; Reduviidae, Subfamily;Triatominae. There are 5 tribes of kissing bugs including Alberproseniini, Bolboderini, Cavernicolini, Rhodniini, and Triatomini (Justi et al., 2017). There are 18 Triatomini genera (Da Rosa et al., 2017), two of which are within the United States, *Paratriatoma* and *Triatoma*. Of these two genera, 11 species occur within the United States.

1.1.3 Identification

They are true bugs belonging to family Reduviidae, which means they are closely related to stink bugs (Pentatomidae), boxelder bugs (Rhopalidae), and leaf-footed bugs (Coreidae), none of which transmit the parasite. However, several key features differentiate them from many look-alike bugs:

1) Coloration. Kissing bugs are black or dark brown over the majority of their bodies.

2) Stripes. Most species found in the U.S. have red, orange, or yellow stripes around the outer edges.

3) Shape. Kissing bugs have a narrow 'shoulder' region, which is distinct from the head.

4) Mouthparts. The mouthparts of a kissing bug are long, straight, and thin, rather than curved and thick.

5) Head. The head is long and thin in comparison to other bugs, which can be short or stout in appearance.

1.1.4 Life Cycle

Triatomines undergo incomplete (hemimetabolous) metamorphosis, in which there are three life stages: egg, nymphs, and adult, with 5 nymphal instars. The entire life cycle of US species of kissing bugs ranges from 1 year to 2 years, depending on species, and duration is not known for all species. *Triatoma rubida, T. lecticularia, T. gerstaeckeri, T. protracta* all take one year to complete their life cycle, while *T. recurva* and *Paratriatoma hirsuta* take two years to complete their life cycle (Lent et al., 1979; Usinger R., 1944; Brunson K., 1959).

In contrast to some triatomine species in Central and South America, which lay eggs in clusters cemented to substrates, the species in the United States lay eggs singly and loosely. The eggs are approximately .5 mm to 2 mm depending on species and are typically glossy white when laid but slowly develop a pink/grey hue before hatching. Triatomines development is highly influenced by environmental conditions, such as temperature, humidity, and light exposure (Lent et al., 1979; Usinger R., 1944). The female kissing bug will begin laying eggs 10 to 30 days after copulation (Usinger R., 1944). An adult female can lay from 500-1,000 throughout the course of her life (Lent et

al., 1979; Usinger R., 1944). Unfertilized females can also lay eggs, although few and infertile.

Of the eleven species of triatomines that occur in the USA, nine have been observed to natural carry the parasite *T. cruzi.* *Paratriatoma hirsuta* and *Triatoma incrassata* are the only species in the United States to not have been observed to be naturally-infected with *T. cruzi*. However, this may also be due to small sample sizes, since *P. hirsuta* has been experimentally infected with *T. cruzi*. All nymphs are hatched uninfected, but may acquire *T. cruzi* infection upon feeding on an infected host as a nymph or adult, and infection of the insect is thought to be lifelong.

1.1.5 Behavior

Kissing bugs are primarily nocturnal and seek hosts during this time. During the day, kissing bugs hide in cracks, crevices, and under debris in order to avoid being detected. Kissing bugs are attracted to light, heat, carbon dioxide, and certain pheromones. Triatomines will associate with animal nests or near blood sources as adults and nymphs, with adults being capable of longer distances of host seeking. Adults can travel long distances during breeding seasons depending on species. Some triatomines have been documented to travel distances up to 6 kilometers (Ekkens, 1981).

1.1.6 Feeding

Kissing bugs feed on blood throughout all life stages, including both males and females. Bugs are opportunistic or generalist feeders that feed on a wide variety of diverse wild and domesticated animals including; humans, domesticated animals, and wild mammals, reptiles, and birds. Examples of blood meals include frogs, rats, dogs, exotic zoo animals (Curtis-Robles et al., unpublished data). Many studies have

documented *T. protracta* in wood rat nests, where the bugs and rats have a co-existence. Depending on life stage, species, and sex, the time for the completion of the full blood meal can range from several minutes to approximately an hour. Some species defecate while feeding; this increases the risk of *T. cruzi* transmission in cases where the feeding bug is infected.

Importantly, the parasite is not spread via the bite of a kissing bug, but rather spread through the infected feces of the kissing bug. The bite of a kissing bug is generally painless but can in some cases cause severe reactions occur leading to anaphylaxis. There have been two recorded deaths due to anaphylactic reactions to kissing bug bite, one being in the United States (Klotz et al., 2016; Vecchio et al., 2004; Teo et al., 1973). Kissing bites usually occur at night, while the victim is at rest or while sleeping.
As kissing bugs progress through their life cycle, they accumulate risk of infection by feeding on multiple hosts. Nymphs are therefore less likely than adults to be infected with *T. cruzi*. This is important to note in rare cases of nymphs being found in human housing in the US. There are cases when a gravid female entered the house, laid eggs, and the nymphs hatched and fed on someone before they were discovered. Two things are important in these cases: 1) the parasite is spread through the feces, not the bite; 2) parasite transmission does not occur from adults to eggs (i.e., no transovarial transmission), therefore newly molted nymphs are uninfected and pose no risk of transmission.

1.1.7 History in the United States

Kissing bugs have been documented in the US since at least 1859, when Carl Stål described the first species in the United States.

Species	Taxonomist	Year Published*	Origin (from original descriptions)
T. gerstaeckeri	Stål	1859	Texas, USA
T. incrassata	Usinger	1939	Valley of Mexico, Mexico 1929, Sonora, Mexico 1939
T. indictiva	Neiva	1912	Arizona, USA
T. lecticularia	Stål	1859	Carolina (not specified whether NC or SC) and Georgia, Tennessee, Texas, USA
T. neotomae	Neiva	1911	Brownsville, TX
T. protracta	Uhler	1894	San Diego, and Monterey County, Santa Clara County, California, USA,
T. recurva	Stål	1868	Arizona, USA
T. rubida	Uhler	1894	Cabo San Lucas, Mexico
T. rubrofasciata	DeGeer	1773	"Indies"
T. sanguisuga	Leconte	1855	Georgia, USA
Paratriatoma hirsuta	Barber	1938	Arizona and California, USA

Table 1. United States species described via publication date and locality found.

*may be different than year specimen was collected, for example *P. hirsuta* was described from specimens collected in 1921, 1929, and 1935

1.2 *Trypanosoma Cruzi* Biology

Trypanosoma cruzi is found throughout the Americas, ranging from the southern US down into northern Chile/Argentina. Although other species of trypanosomes are found in other areas of the world, *T. cruzi* in natural cycles is limited to the Americas. *Trypanosoma cruzi* in vectors and non-human animals has been found in 30 total states within the United States (Table 1).

1.2.1 History, with Attention to the United States

Dr. Carlos Chagas, a Brazilian clinical biologist working in rural Brazil, discovered the parasite *T. cruzi* in 1909. *Trypanosoma cruzi* was named in honor of his mentor, Dr. Oswaldo Cruz, the director of the national research institute in Brazil. Dr. Chagas discovered the parasite in kissing bugs that the local people noted as having bitten them in the night (Carlos C., 1909). He soon discovered that this parasite could cause disease in people and animals. Dr. Chagas' findings were published in 1909, and other scientists soon started to investigate whether this parasite was found in other areas as well (Carlos C., 1909). In 1916, two researchers in California—Kofoid and McCulloch—found a very similar-looking parasite in kissing bugs from a wood rats nest in southern California (Bern et al. 2011) (Kofoid et al., 1916). At first, they thought they had found a similar, but not the same, species of trypanosome, because the rats in the nest did not appear to be infected. Several years later, after finding that the parasite did cause infection, they realized that the parasite was in fact *T. cruzi*. Subsequently, many reports of *T. cruzi* infection in bugs and wildlife across the US were reported by researchers (Bern et al., 2011). The first described human case of Chagas disease in the US was in 1955 (Bern et al., 2011). A husband-wife team of pediatricians diagnosed Chagas disease

in a 10-month infant from Corpus Christi, Texas. In 1956 another case was described in a patient from Bryan, Texas (Anonymous, 1956). The first description of *T. cruzi* infection in dogs occurred in 1977 (Williams et al. 1977).

1.2.2 Life Cycle

Trypanosoma cruzi undergoes several transformations both in the insect vector and mammalian host. Once the trypomastigote stage enters a mammalian host, the parasite seeks a host cell to begin proliferation and differentiation. *Trypanosoma cruzi* can infect many different mammalian host cells; perhaps most notable is cardiac tissue. The protozoan then begins to differentiate from trypomastigotes to amastigotes and begins proliferation via binary fission within the cell. Prior to cytolysis, amastigotes transform to trypomastigotes, and after the cell ruptures, may spread to neighboring cells or begin circulation via lymphatic or blood vessels to other tissues within the host (Cardonna et al., 2011). Trypomastigotes circulating in mammalian blood may be ingested by kissing bugs when blood feeding on hosts. Within the kissing bug vectors the trypomastigotes differentiate into epimastigotes, while traveling through the intestinal tract of the bugs. The epimastigotes attach to the walls of the intestines and begin proliferation via binary fission. However, once in the hindgut the epimastigotes begin to differentiate into trypomastigotes again and are passed in the insect feces. These trypomastigotes may infect a mammalian host via mucousal membranes, lesions, or bite site in the epidermal layer.

1.2.3 Transmission Routes

T. cruzi can be transmitted several ways. Stercorarian transmission is the primary form of transmission via rubbing in the fecal matter of infected kissing bugs into the bite

site, skin abrasions, or mucosal tissue. Oral transmission, from eating or drinking contaminated foods, has been documented in several cases. Oral transmission through ingestion of infected triatomines may be an important mode of transmission to wildlife and domestic animals. Congenital transmission has been shown to occur in dogs and humans, although the degree to which infected mother pass infection to their offspring is unknown. Finally, there have been documented cases of *T. cruzi* transmission through infected blood transfusions and organ transplants.

1.3 Chagas Disease

1.3.1 Pathogenesis in Vertebrates

T. cruzi infections are dependent of many different factors within the host-parasite interactions that result in cardiovascular damage. Host immune response is a major factor in the progression of Chagas disease in canines and humans alike. Strain type also plays a role on how a host is affected by *T. cruzi*. Different *T. cruzi* strain types have illustrated different inflammatory responses in canines in both acute and chronic infections (Kjos et al., 2008). In human acute cases, microangiopathy and ischemia has been observed in cardiovascular damage.

1.3.2 Animal Epidemiology

Chagas disease has recently gained significant attention within the United States in companion and working animals, especially in domesticated canines. There are many ecological factors that play a role in domestic transmission in humans and animals. Many studies have illustrated that human infections, are associated with pet or companion animal infections. This illustrates that dogs may increase the chance of

human infections due to kissing bug populations increasing when dog hosts are more readily available (Tenney et al., 2014). This poses a threat to human cases in domestic transmission. Stercorarian transmission is most common for humans but other infections have occurred from oral ingestion (Nobrega et al., 2009) (Montgomery et al., 2014).

Clinical signs in dogs include: lethargy, decreased appetite, distended abdomen "ascites") cardiac arrhythmia, dilated cardiomyopathy (Tenney et al., 2014).
Studies have shown that opossums, raccoons, and wood rats have been known to ingest infected bugs themselves, which is potentially a route for wild and domesticated dogs transmission (Roellig et al., 2009). Another form of transmission of Chagas in canines is transplacental and transmammary transmission. In canines, this transmission is observed in the acute stage and typically will result in a puppy's death within one year.
Seroprevalence in dogs in certain areas of Central American countries have reported up to 21% (Estrada-Franco et al., 2006). In domesticated dogs, acute and chronic Chagas disease has been observed in 16 states within the United States (Kjos et al., 2014). The first canine cases (9) were observed in eight different counties within Texas in 1972 (Williams et al., 1977). Working and sporting dogs have been observed with higher risk of Chagas infections due to life style factors such as outdoor kenneling or being in sylvan areas where the vector populations can be more abundant (Snowden et al., 2012).
Canine Chagas disease, as in humans, exhibits both an acute and chronic phase with heart disease being the most prominent manifestation of both stages. Within weeks of exposure to the parasite, the body executes a cascade of events within the immune system in an effort to eliminate the parasite from the body (Cardonna et al., 2011). Due to severe inflammation, dogs may exhibit fever, lethargy, vomiting, swollen lymph nodes, collapse,

swelling in the abdomen or belly, seizures, and in some cases death (Curtis-Robles et al., 2016).

Following the acute phase, most dogs enter a prolonged chronic phase where symptoms are silent, however, the parasite maintains residency in both heart and adipose tissues, as well as inside the immune system to avoid detection by the body. It is this slow, steady, progressive inflammation and infection that cause the heart cells to slowly be destroyed, and to be replaced with nonfunctional scar tissue (Curtis-Robles et al., 2016). Over the years, this may lead to bouts of weakness, loss of appetite, collapse, weight loss, or dogs may acquire the most severe form of the disease, Chagas cardiomyopathy (Curtis-Robles et al., 2016). Some dogs never develop Chagas related symptoms, however, 25% of dogs experience complications including heart rhythm abnormalities that can cause weakness or sudden death, a large, weak heart, or lack of energy. Infected pregnant females can also pass the infection to their offspring, contributing to puppy mortality.

1.3.3 Treatment Options

There are only two antiparasitic drugs for *T. cruzi* infections: benznidazole and nifurtimox. The U.S. Food and Drug Administration granted the approval for benznidazole to be used for children with Chagas disease from ages 2 to 12 years of age. Benzindazole is the first treatment approved for use against Chagas disease in the United States. Nifurtimox can only be received through investigational protocols by the Centers for Disease.

1.4 Diagnostics

1.4.1 Microscopy

Historically, the most common method of detection of *T. cruzi* infection was is via direct observation of parasites in a blood smear from an individual suspected to be infected. A blood smear can reveal motile trypomastigotes, and Giemsa stain or other stains may be used and can be identified morphologically.

Importantly, circulating parasites occur for a relatively short time period, during the acute phase of Chagas disease. Infected individuals may have microscopically-detectable levels of parasites also known as parasitemia for only a few weeks. An additional complicating factor is the variety of other trypanosomes with similar morphology, which may be particularly relevant in diagnosis of infection in animals, which are known to harbor a variety of other trypanosome species.

1.4.2 Parasite Culture

Culturing parasites from a blood sample (hemoculture) is relatively sensitive to detect *T. cruzi*. However, cultures require weeks to months of maintenance before *T. cruzi* is detectable. Some difficulty can be had in avoiding bacterial contamination that can overgrow the *T. cruzi*. Further, biosafety requirements reduce the ability of hemoculture to serve as a standard diagnostic method in the US.

1.4.3 Serology

Serology, or antibody detection, is the main method of diagnostic testing of humans and dogs in the United States. When exposed to *T. cruzi*, most healthy individuals will develop antibodies against *T. cruzi*. These specialized proteins help protect the individuals and fight infection. Antibodies against *T. cruzi* may help the

individual manage the infection and resist future infections. Antibodies can be found in circulating blood, and therefore a blood sample can be tested to determine presence of antibodies, and indication of whether an individual has been exposed to the parasite. Serologic platforms used for human testing include indirect fluorescent antibody (IFA) testing, ELISA, and precipitation assays. Additionally, a number of rapid immunochromatographic 'dipstick' tests have been developed and are available for research purposes, with a single rapid test approved by FDA for use in the US in 2017. For dogs, a major veterinary diagnostic laboratory in the south offers IFA testing, and there are few other options available, especially for non-canine species.

1.4.4 Xenodiagnosis

Because *T. cruzi* is a life-long infection, but with extremely low numbers of circulating parasite, it is not always possible to see parasite on blood smear samples from suspected positive individuals. Historically, xenodiagnosis was used to diagnose *T. cruzi* infection. In this method, uninfected kissing bugs are allowed to feed on suspected infected individuals. The nymphs have been raised in laboratory environment and known to be free of *T. cruzi* infection. At periodic intervals after feeding, the feces of the bugs are checked for *T. cruzi*. Presence of *T. cruzi* in the nymph feces is indication that the individual upon whom they fed is infected. This technique was not 100% sensitive, and generally several nymphs were fed on the individual in order to increase chances of correct diagnosis. This method has largely been replaced with PCR-based techniques.

1.4.5 PCR-Based Techniques

Since soon after its development in the late 1980s, polymerase chain reaction (PCR) has been used for detection of *T. cruzi* DNA in samples. DNA extracted from

potentially infected samples undergoes a series of heating/cooling cycles with *T. cruzi*-specific primers that result in the amplification of many millions of copies of *T. cruzi* DNA from as few as one starting copy in the infected sample. The amplified copies can be visualized to confirm presence or absence of *T. cruzi* DNA in a sample. The main PCR targets for amplification of *T. cruzi* DNA are nuclear targets, which target DNA sequences in the nucleus of the parasite, and minicircle targets, which target the DNA sequences in the kinetoplast structure of the parasite. Some PCRs target very conserved regions of DNA that are the same for all *T. cruzi* strains. Other PCRs target regions of DNA that are variable depending upon the strain type of DNA, which can offer additional valuable information about a sample. PCR is useful for detecting acute cases, but may not be useful for determining if an indeterminate or chronic infection exists. Serology would be better in that case.

One caveat of PCR is that because only a very small part of the DNA is being amplified, we can't be sure whether parasite detected is whole, viable parasite. Some researchers will therefore amplify from various targets to offer more evidence that a whole parasite is in the sample.

1.5 Surveillance

1.5.1 Kissing Bug Collection Techniques

In contrast to regions of South and Central America, where triatomines in the domestic setting are the main concern, the situation in the US is decidedly different. Kissing bugs in the US generally occur in the peridomestic and sylvatic environments. While domestic searching in Central and South America may be successful during the

day, sylvatic kissing bug activity in the US is most easy to catch them at night. There are many different methods of collecting kissing bugs but there is no perfect method of capturing the illusive insects. The most commonly used method is light sheet traps (black light or mercury vapor lights), which are cheap, easily transported, and can be effective in areas common with kissing bug populations. In general most insects are attracted to different light spectrums, in the case of Triatomines, black light is preferred (Figueiras et al., 1994) (Ekkens D., 1981). When beginning this method begin by using white bed sheets tied on an object approximately four to five feet from the ground with a black light in front of the white sheet, which will disperse the light out towards targeted area. When using this method targeting a specific area is important, since kissing bugs are not general out in plain sight and are primarily nocturnal it is important to begin when light is absent and placing sheet/light on higher surfaces, overlooking brush piles, debris piles, cacti grouping (where rodents may possible be observed). Other methods that have provided sufficient results are carbon dioxide based traps, which can also be modified for personal trapping efforts.

1.5.2 Human Serosurvey

As of 2013, *T. cruzi* infection in humans is a notifiable disease in Texas, which joined three other states (along with Arizona, Massachusetts, and Tennessee) in required reporting. The disease is not currently a national notifiable disease, although estimates of infection range from ~300,000 to approximately 1 million infected in the US. These cases are mainly people who have lived for extended periods of time in Central and South America.

1.6 Integrated Vector Management

Integrated Pest Management (IPM) is a pest control strategy based on pest prevention through various integrated methods, utilizing pesticides as a method when necessary. IPM utilizes three broad procedures to safely eliminate pest threats: inspection, monitoring, and reporting. Inspection allows the homeowner or concerned individual to determine the need for IPM through identification of the pest, monitoring the size of the pest population, investigating additional areas for risk of disease transmission, and monitoring control measures. All inspection and monitoring information as well as control measures should be recorded and updated. IPM was developed primarily for agricultural pests, but the principles of IPM provide a comprehensive framework for addressing problems related to bloodsucking arthropod vectors, termed Intergrated Vector Management.

For a kissing bug concern in the home, it is important that suspect insects are identified by a trained entomologist or pest control professional. Multiple resources are available throughout Texas to help identify potential kissing bugs; for example, our Kissing Bug Citizen Science program (kissingbug.tamu.edu) offers a free identification and testing service, starting with a photograph uploaded to the website. Determining the risk for humans or animals in the home requires knowing where the bugs are accessing potential blood meals through inspection i.e. animals outside the home or humans/pets inside the home.

There are multiple types of control measures recommended for kissing bug infestations. The first is physical control which focuses on eliminating bug resting places by keeping them away from potential blood sources inside and outside of the home. A

large part of physical control is housing/building structure: all windows and walls inside the home should be screened or sealed properly; all cracks or fissures on the outside of the home or roof should be properly sealed. All animal shelters should not be directly adjacent to the home, food sources should be protected from rodents, and all animal cages or bedding should be routinely cleaned. Large debris piles or stacks of wood should be removed and eliminated from the backyard. Lastly, entomological surveillance should be conducted routinely throughout the home to prevent future infestations.

Chemical control is another proven method of control for kissing bugs. If kissing bug nymphs are found inside the home during inspection, it is very possible that the home was colonized, and chemical control is strongly recommended. Entomological expertise is highly recommended through a pest control company. Synthetic pyrethroids are chosen for kissing bug control because they have low toxicity to humans, strong triatomicidal effects, and high residual repellency. They are applied using a manually-operated variable pressure sprayer onto walls in and around the home or structures where kissing bugs are present and hiding, depending on the level of infestation.

1.6.1 Habitat Source Reduction/Reservoir Reduction

The most efficient method of controlling Chagas disease in domestic and urbanized settings is by reducing the habitats associated with kissing bugs. One way this can be performed is by cleaning brush piles, woodpiles near houses, and other debris that reservoirs such as rodents can nest in.

Woodrat nests are a known source of kissing bugs, so we expect that general elimination of animal dens/nests/holes would eliminate sources of kissing bugs. It is generally accepted that turning outdoor lights off during the night will reduce the

attraction by kissing bugs, which may orient their flight toward lights. However, some wavelengths of light may be attractive and others deterrent; more research is needed in this area.

1.6.2 Physical Barriers

Physical barriers are a good way to control kissing bugs from feeding or associating with mammalian hosts. The best way to prevent kissing bug invasion is by having properly sealed homes. Windows that can be sealed and have screens on the outside while still allowing air to pass through as well as doors with weather stripping or blockers will aid in kissing bug house invasions. Some of these can be expensive but are effective ways of avoiding triatomine invasion. Another way to prevent exposure is by using mosquito bed nets that are properly tucked under a mattress, overlapped at opening and clipped with material such as clothespins so that the net is completely sealed. If pets are left outside at night such as domesticated dogs proper housing should be installed that are sealed, therefore alleviating triatomine contact and population growth. Using caulk or foaming spray to seal cracks or crevices around complexes can also be an efficient and inexpensive way in preventing kissing bug contact.

CHAPTER II

DIFFERENTIATING NORTH AMERICAN TRIATOMINES FROM OTHER INSECT SPECIES

2.1 Synopsis

Triatomines have been a major issue for professionals and laypersons to identify, causing some misidentifications and unnecessary anxiety to concerned individuals. Such worry is exacerbated when the media features press about Chagas disease yet shows a photo of an insect that is not a kissing bug. We present a novel photographic key for the United States kissing bugs and misidentified non-kissing bugs commonly observed, from taxa within order, family, subfamily, tribes, to genera and species. Descriptions of each taxon are illustrated through a comprehensive dichotomous key based on morphological ques from peer-reviewed publications.

2.2 Introduction

Triatomines transmit *Trypanosoma cruzi* the etiological agent of Chagas disease. Within the subfamily there are 18 genera of which there are 152 species, most of which capable of transmitting *T. cruzi* (Da Rosa et al., 2017) (Galvao C., 2014) (Mendonca et al., 2016) (Poinar G., 2013) (Souza et al., 2016). In the United States, the genera *Paratriatoma* and *Triatoma* exist, which include *P. hirsuta, T. gerstaekeri, T. incrassata, T. indictiva, T. lecticularia, T. neotomae, T. protracta, T. recurva, T. rubida, T. rubrofasciata,* and *T. sanguisuga* (11 species) with only *P. hirsuta* and *T. incrassata* not shown to be naturally infected with *T. cruzi* (Lent et al., 1979) (Usinger et al., 1944).

Based on previously published data (Bern et al., 2011) (Curtis-Robles et al., 2015) (Lent et al., 1979) (Schmidt et al., 2011) (Usinger R., 1944), in combination with archived specimens at the Texas A&M University Insect Collection, and submissions to our active citizen science program to solicit kissing bugs from the public, I compiled state-level reports of all kissing bugs species in the US. I used these data to create a contemporary state-level may of reported triatomine distribution within the United States (Figure 1; Table 2).

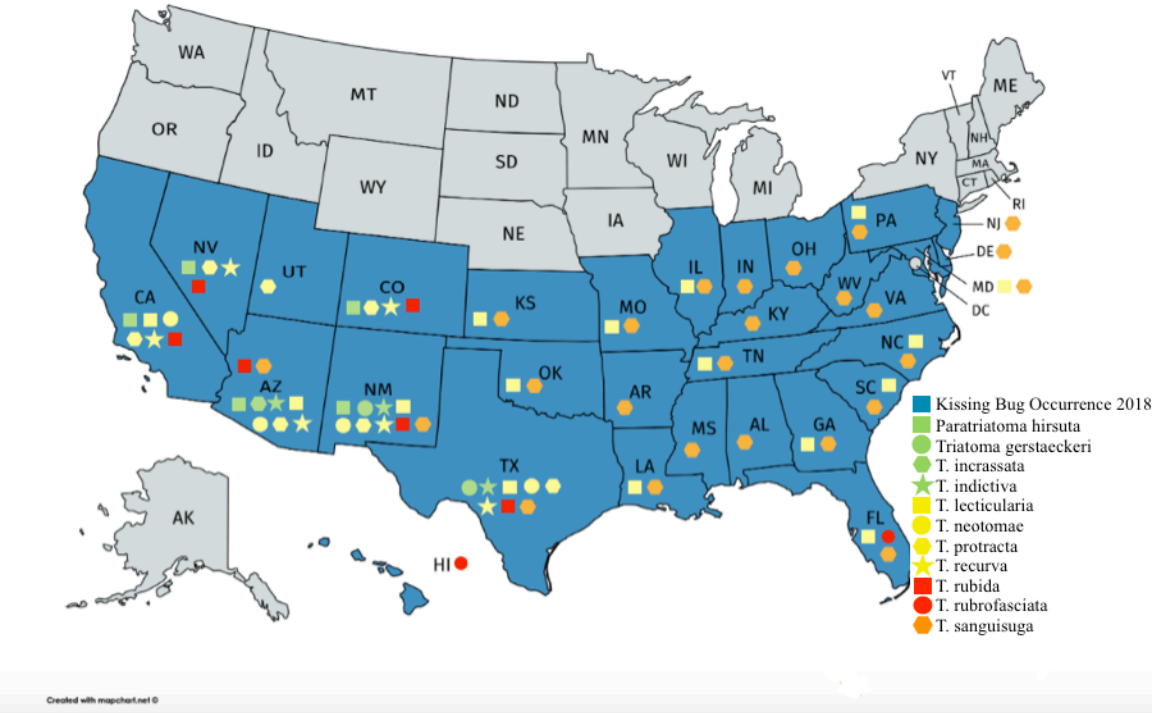

Figure 1. (https://mapchart.net/usa.html) Distribution of United States kissing bug species by state. State occurrence (Blue), Species as follows; *Paratriatoma hirsuta* (Green Square), *Triatoma gerstaeckeri* (Green Circle), *T. incrassata* (Green Hexagon), *T. indictiva* (Green Star), *T. lecticularia* (Yellow Square), *T. neotomae* (Yellow Circle), *T. protracta* (Yellow Hexagon), *T. recurva* (Yellow Star), *T. rubida* (Red Square), *T. rubrofasciata* (Red Circle), and *T. sanguisuga* (Orange Hexagon)

State/KB spp.	AL	AR	AZ	CA	CO	DE	FL	GA	HI	IL	IN	KA	KY	LA	MD	MS	MO	NV	NJ	NM	NC	OH	OK	PA	SC	TN	TX	UT	VA	WV
P. h.		X		X	X													X		X										
T. g.																				X							X			
T. ic.			X																											
T. id.			X																	X							X			
T. l.			X	X			X	X		X		X		X	X		X			X	X		X	X	X	X	X			
T. n.			X	X																X							X			
T. p.			X	X	X													X		X							X	X		
T. re.			X	X	X													X		X							X			
T. rd.			X	X	X													X		X							X			
T. rb.							X		X																					
T. s.	X	X	X			X	X	X		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X

Table 2: Kissing bug species by state of occurrence. Species as follows; *Paratriatoma hirsuta* (P. h.), *T. gerstaekeri* (T. g.), *T. incrassata* (T. ic.), *T. indictiva* (T. id.), *T. lecticularia* (T. l.), *T. neotomae* (T. n.), *T. protracta* (T. p.), *T. recurva* (T. re.), *T. rubida* (T. rd.), *T. rubrofasciata* (T. rb.), and *T. sanguisuga* (T. s.), with abbreviations of states with kissing bugs.

Within the United States, *T. cruzi* transmission is somewhat rare for humans but does occur frequently in domesticated and wild animal species (Bern et al., 2011) (Curtis-Robles et al., 2016) (Tenney et al., 2014) (Klotz et al., 2014). Chagas disease in the United States has gained significant recognition recently, resulting in a concerned public. Misidentifications of the true kissing bug vectors have been publicized and commonly occur. Unfortunately people without entomological expertise have difficulty in distinguishing these vectors from other common look-a-likes. With only peer-reviewed morphological information currently available consisting of only single highly-technical entomological keys that can only be understood by entomological professionals, a need for photographic information of the morphology is required (Lent et al., 1979). This dichotomous key will assist in both individuals without entomological expertise as well as professionals within human and animal healthcare, public health, educational leaders can easily distinguish these vectors from non-vectors.

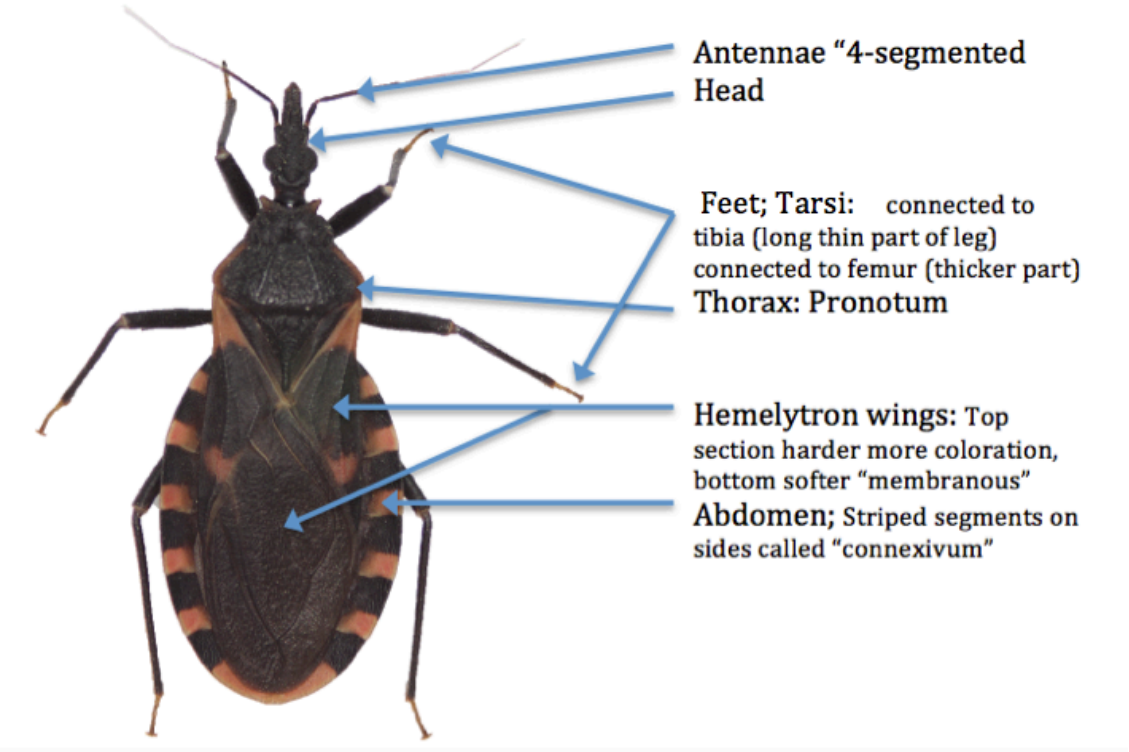

Figure 2. *Triatoma sanguisuga* (Leconte) (Hemiptera: Reduviidae) dorsal morphology.

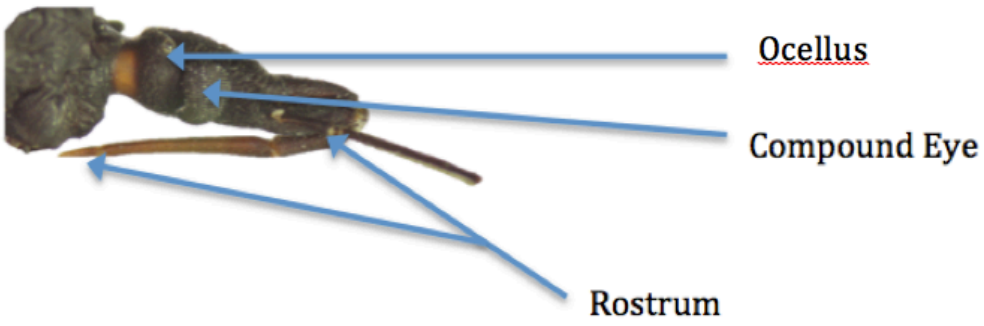

Figure 3. *Triatoma indictiva* (Neiva) (Hemiptera: Reduviidae) lateral head morphology.

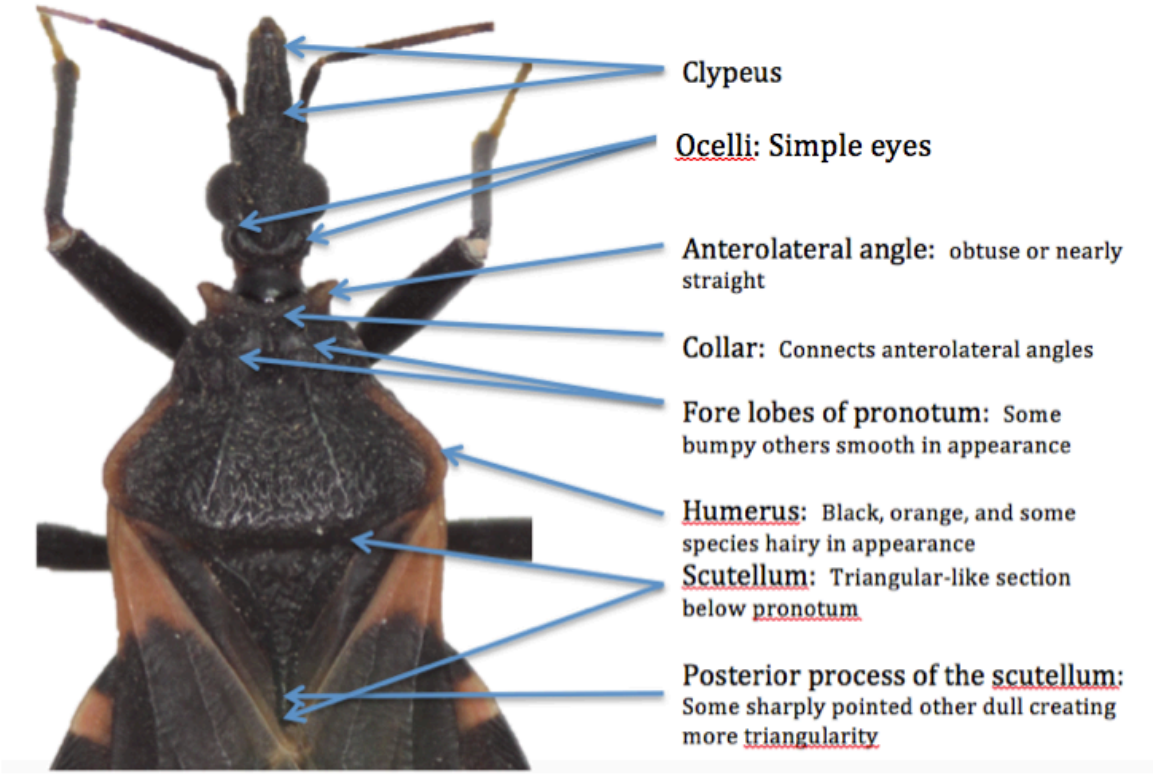

Figure 4. *Triatoma sanguisuga* (Leconte) (Hemiptera: Reduviidae) dorsal head and thorax morphology.

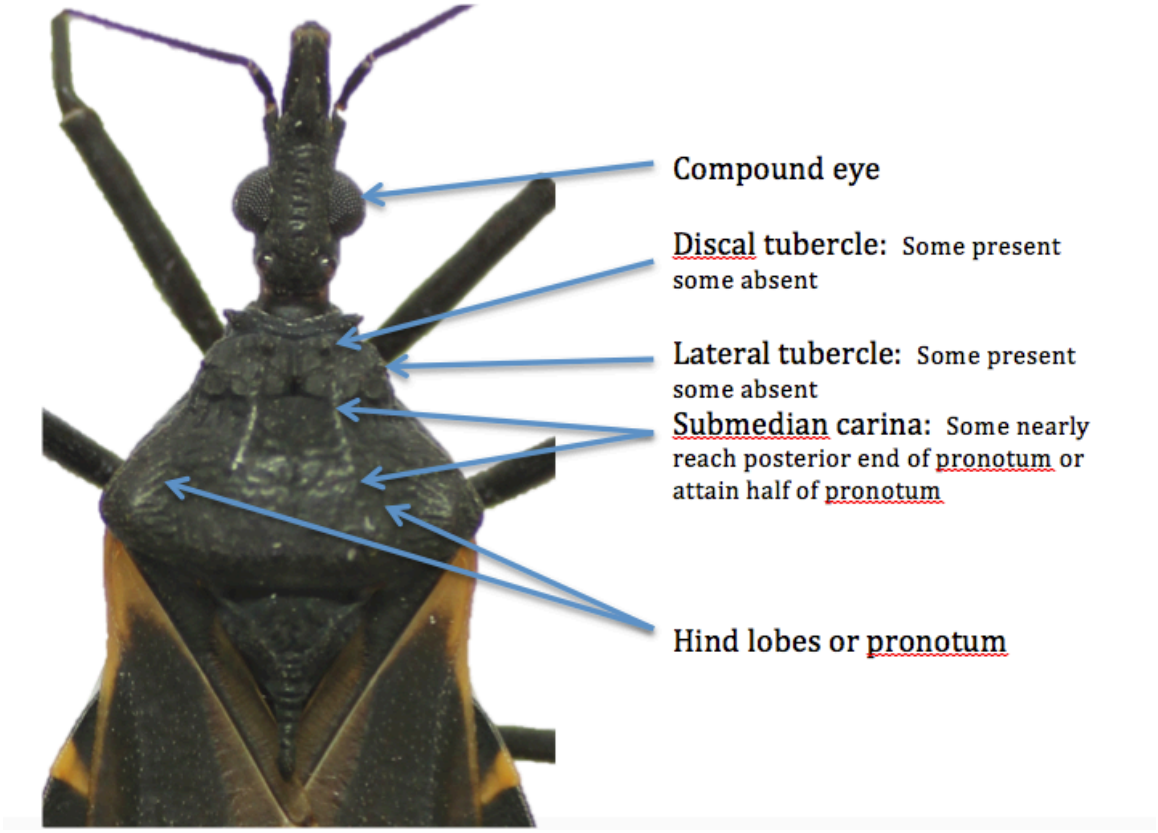

Figure 5. *Triatoma gerstaeckeri* (Stål) (Hemiptera: Reduviidae) dorsal head and thorax morphology.

2.3 Differences between Hemiptera and other Orders and Suborders within Insecta (Triplehorn et al., 2005) (*denotes kissing bug taxa)

Hemipterans have hemelytra wings, which only have partially sclerotized forewings distinct from other insects orders such as elytra-fully sclerotized forewings, fully membranous wings, or wingless: Blattodea-now encompassing Isoptera-Termitoidea, Coleoptera, Dermaptera, Diptera, Embiidina, Ephemeroptera, Grylloblattodea, Hymenoptera, Lepidoptera, Mantodea, Mecoptera, Microcoryphia, Neuroptera, Odonata, Orthoptera, Phasmatodea, Phthiraptera, Plecoptera, Psocoptera, Siphonaptera, Strepsiptera, Thysanoptera, Thysanura, Trichoptera, and Zoraptera.

Hemipterans are also distinguished by a rostrum, which, unlike other insects with piercing/sucking mouthparts, is segmented and more heavily sclerotized. Hemipterans also have a life cycle known as hemimetabolous or incomplete metamorphosis-"eggs, nymphal stages, adults", which also includes orders: Orthoptera, Mantodea, Dermaptera, Odonata, Phasmatodea, Phthiraptera, Ephemeroptera, Plecoptera, Grylloblattodea, and some Blattodea. Other insect orders have holometabolous or complete metamorphosis life cycles -"eggs, larval stages, pupae stage, adults" and ametabolous or no metamorphosis-"eggs, no changes within nymphal stages, adults." Within Hemiptera there are three suborders Auchenorrhyncha, Heteroptera, and Sternorrhyncha. Heteroptera can be distinguished between the other suborders by the placement of the rostrum on the body. Heteropterans, rostrum arises at the front of the head, where Auchenorrhynchas' rostrum arises at the base of the head and Sternorrhynchas' rostrum arises nearly in between the forelegs.

2.4 Dichotomous Key to Family for Heteroptera with Similar Morphology to Reduviidae (Triplehorn et al., 2005)

1.	Antennae 4-segmented	2
1'.	Antennae 5-segmented	Pentatomidae
2(1).	Tarsal 1-segmented	Nabidae
2'.	Tarsal 2-3 segmented	3
3(2').	Tarsal 2-segmented	Aradidae
3'.	Tarsal 3-segmented	4
4(3').	Prosternal groove present, rostrum 3-segments	Reduviidae*

4’. Prosternal groove absent, rostrum 3-4 segments 5

5(4’). Scent glands between middle and hind coxae 6

5’. Scent glands absent Rhopalidae

6(5). Ocelli present 8

6’. Ocelli absent 7

7(6’). Rounded apically on pronotum Largidae

7’. Margined apically on pronotum Pyrrhocoridae

8(6). 4-5 simple veins arising from base of hemelytra membrane Lygaeidae

8’. Many veins arising from base of hemelytra membrane 9

9(8’). Pronotum longer and wider than head Coreidae

9. Pronotum as long and wide as head Alydidae

2.5 Dichotomous Key to Subfamilies for Reduviidae with Similar Characteristics to Triatominae (Weirauch et al., 2014)

1. Labium visible before 3-segmented rostrum 2

1’. Labium reduced before 3-segmented rostrum Hammacerinae

2(1). Quadrate, pentagonal, hexagonal cell on cubitus present, basal tooth present underneath scape on tarsal claw 3

2’. Quadrate, pentagonal, hexagonal cell on cubitus absent, basal tooth absent underneath scape on tarsal claw 4

3(2). Antennal pedicel folded underneath scape, usually pentagonal or hexagonal cell on cubitus Stenopodainae

3’. Antennal pedicel does not fold underneath scape, usually quadrate cell on cubitus Harpactorinae

4(2’). Rostrum segment-2 thin, straight, and usually elongated in comparison to segments-1 and 3 Triatominae*

4’. Rostrum segment-2 thick, curved, and equal to or shorter than segments-1 and 3 5

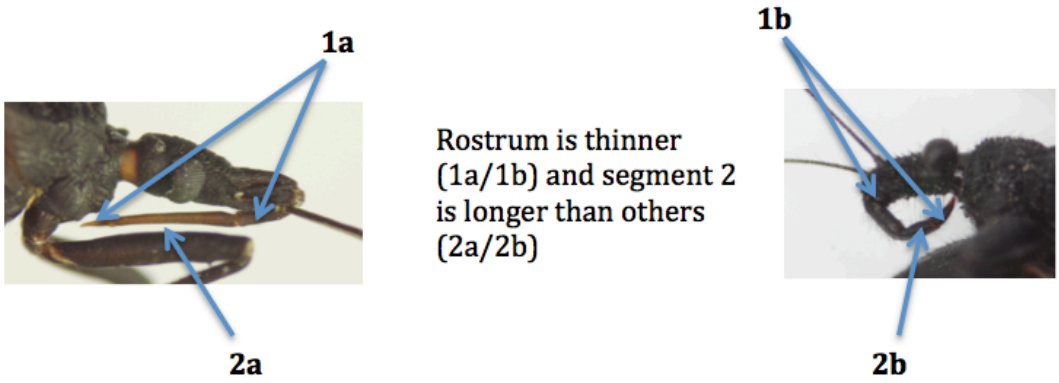

Figure 6. Left, *Triatoma indictiva* (Neiva) (Hemiptera: Reduviidae); Right, *Microtomus purcis* (Drury) (Hemiptera: Reduviidae) lateral comparison of rostral appearance.

5(4’). Transverse sulcus behind the middle of the pronotum, forecoxa flattened laterally Peiratinae

5’. Transverse sulcus in front of the middle of the pronotum, forecoxa rounded laterally 8

6(5’). Antennal flagellomeres subdivided into pseudosegments, scutellum prongs laterally projecting and lacking median tip Ectrichodiinae

6’. Antennal flagellomeres are not subdivided into pseudosegments, scutellum no prongs laterally projecting and median tip 9

7(6’). Head usually elongate; if short, eyes reniform and not strongly pedunculate and not covering entire lateral surface of head Reduviinae

2.6 Dichotomous Key to Tribes for Triatominae with Similar Characteristics to Triatomini (Lent et al., 1979)

1. Ocelli not elevated, situated at level of integument, inconspicuous among coarse head granules or in or very close to interocular sulcus 2

1’. Ocelli situated on distinct elevations on disc of postocular portion of head 3

2(1). Head elongated, head not strongly convex dorsally; maxillary plate projecting beyond apex of clypeus; interocular sulcus obsolete; corium with distinct veins; integument heavily granulose Bolboderini

2 Head ovoid, strongly convex dorsally; maxillary plate small; interocular sulcus strongly backwardly curved; corium lacking venation; integument smooth and/or hairy Cavernicolini

3(1’). Head elongate, antennal insertion located apically of head Rhodniini

3’. Head elongate or short and wide, antennal insertion remote from apex of head 4

4(3’). Head short and wide, hemelytra with small branch connecting basal portion of R-M to Sc, body length equivalent to 5 mm Alberproseniini

4’. Head elongate, branch connecting basal portion of R-M to Sc absent on hemelytra, body length greater than 5 mm Triatomini*

2.7 Dichotomous Key to Genera for Triatomini with Similar Characteristics to Triatoma (Lent et al., 1979)

1. Head strongly convex above; long, curved, semi-erect hairs abundant on head, body and appendages; eyes small; antenniferous tubercles inserted close to anterior margin of eyes; fore femora denticles absent; fossula spongiosa absent; body length 12.5-14.5mm Paratriatoma hirsuta* (Barber)

1'. Head less strongly convex above; some with short hairs on head, body, and/or appendages, if long hairs only on appendages; eyes larger; antenniferous tubercles inserted at or close to middle of anteocular region of head, remote from eyes; fore femora denticles present in most species two or more; fossula spongiosa present or absent; body length 9.5-42 mm 2

2(1'). Fore femora not denticulate; ventral connexival plates not perceptible, conspicuous longitudinally, pleated membrane connecting dorsal connexival plates to urosternites; body length 33-42 mm Dipetalogaster

2'. Fore femora denticulate or not; ventral connexival plates distinct, narrow in some species; sides of abdomen rarely membranous, only in micropterous female, with membrane connecting dorsal and ventral connexival plates; body length rarely 33 mm, most species less than 30 mm Triatoma*

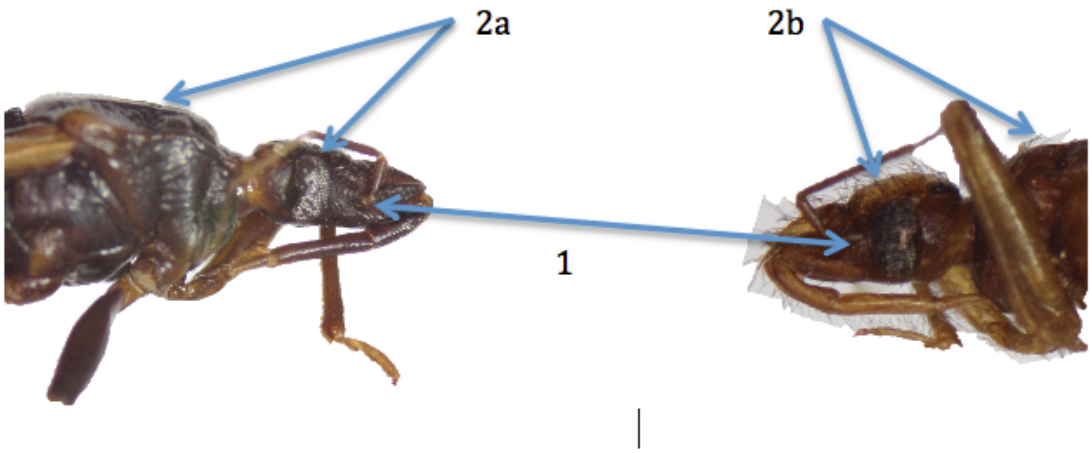

Figure 7. Left, Triatoma neotomae (Neiva) (Hemiptera: Reduviidae); Paratriatoma hirsuta (Barber) (Hemiptera: Reduviidae) head and body morphology comparison. (1) antenniferous tubercles insertion P. hirsuta inserting next to eye in comparison to T. neotomae inserting away from eye. (2a) T. neomtomae nearly hairless in appearance compared to (2b) P. hirsuta with numerous setae "hair-like structures."

2.8 Dichotomous Key to Species for Triatoma Relevant to the Known United States Kissing Bugs (Lent et al., 1979)

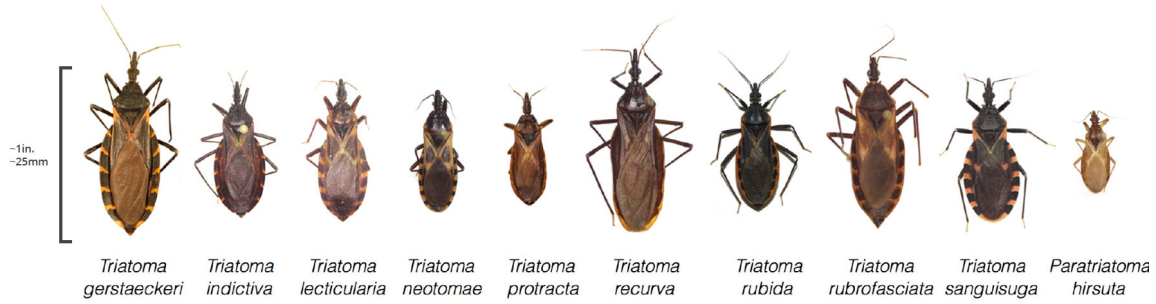

Figure 8. Ten of the eleven species found in the United States.

1. Body clothed with numerous black setae, conspicuous on head, all three segments of rostrum, pronotum and corium; head strongly convex dorsally, especially between eyes; antenniferous tubercles elongate, close to eyes in comparison to other species lecticularia* (Stål)

1'. Body practically glabrous, most short scattered setae; rostrum either entirely glabrous, or with long setae on segments-1, 2, not on segment-3; head not strongly convex above; antenniferous tubercles short, remote from eyes 2

2(1'). Antennal segment-1 attaining or surpassing level of apex of clypeus 3

2'. Antennal segment-1 not attaining level of apex of clypeus 4

3(2). Head and pronotum strongly granulose; anterolateral angles of pronotum prominent, oblique; posterior process of scutellum short rubrofasciata* (DeGeer)

3'. Head and pronotum not granulose; anterolateral angles of pronotum short, obtuse; apical process of scutellum elongate rubida* (Uhler)

4(2'). Postocular region with sides straight, subparallel; rostrum practically glabrous; larger more than 24 mm, rarely only 23 mm 5

4’. Postocular region with sides distinctly convex; rostrum glabrous or with numerous long setae on segments-2, 3; smaller length of 24 mm or less 6

5(4). Connexivum dorsally with light-colored area extending along entire lateral border; corium of uniformly dark color recurva* (Stål)

5’. Connexival segments black, narrowly marked with yellow along posterior fourth or third adjacent to intersegmental suture; corium dark, with base and subapical spot yellowish gerstaeckeri* (Stål)

6(4’). Anterior lobe of pronotum with the usual ridges without tubercles; anterolateral angles obtuse, not prominent; rostrum with abundant long hairs on segments-2, 3 7

6’. Anterior lobe of pronotum with distinct discal tubercles; anterolateral angles of pronotum pointed, prominent; rostrum practically glabrous 9

7(6). Upper and lower surface of connexival segments marked transversally with dark and light; abdomen conspicuously flattened ventrally along middle neotomae* (Neiva)

7’. Connexivum without transversal markings; abdomen convex below 8

8(7’). Base of clypeus conspicuously swollen, strongly convex in lateral view; fossula spongiosa present on fore tibia in both sexes; connexivum dorsally light colored on outer portion, dark on inner portion incrassata* (Usinger)

8’. Base of clypeus not strongly swollen, its dorsal surface straight in lateral aspect; fossula spongiosa absent in both sexes; connexivum unicolorous protracta* (Uhler)

9(6’). Pronotum dark, with red or yellowish markings in form of narrow band on collar, on anterolateral angles and sides of pronotum, and on humeri and some portions along hind border of pronotum; dorsal and ventral surface of connexivum with light colored markings occupying from one-fourth to one-half of each segment posteriorly, including

intersegmental sutures, with narrow light-colored area situated behind suture

sanguisuga* (Leconte)

9’. Pronotum uniformly piceous or black; connexivum dorsally and ventrally with faint reddish band on posterior portion of segments adjacent to intersegmental suture

indictiva* (Neiva)

[*]CHAPTER III

CLEAR RESIN CASTING OF ARTHROPODS OF MEDICAL IMPORTANCE FOR USE IN EDUCATIONAL AND OUTREACH ACTIVITIES

3.1 Synopsis

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.

3.2 Introduction

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

[*] Reprinted with permission from "Clear Resin Casting of Arthropods of Medical Importance for Use in Educational and Outreach Activities" by Bejcek, J.R., Curtis-Robles, R., Riley, M., Brundage, A., Hamer, S.A. & G.L. Hamer, 2018. Journal of Insect Science, Volume 18, Issue 2, Copyright 2018 by Entomological Society of America.

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, while specimens in ethanol or those pinned or pointed inevitably become damaged while handling. The embedding of specimens in optically-clear resin is a 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.

3.3 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 hours. 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 x 3.8 x 2 cm) molds of specimens such as ticks, and larger (approximately 5 x 7.6 x 3 cm) molds of specimens such a 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 compromises 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 pre-

sized 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 non-permanent 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.

(1) 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.

(2) First layer of resin. In a chemical fume hood or other well-ventilated area, mix vigorously for one minute: 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, San Leandro, CA). 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 150x15mm petri dishes (Corning™ Falcon™ Bacteriological Petri Dishes with Lid, Corning™ 351058, Corning, NY).

(3) 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 minutes, 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 minutes. When resin begins to thicken, but before it reaches a gelatinous state, set specimens in resin and push down slightly so that the specimen is slightly floating in the resin (Figure 1A).

(4) Second layer of resin. Once the resin has reached a gelatinous state, approximately 5 minutes 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 hours) at room temperature in the fume hood or other well-ventilated area.

(5) 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 (Figure 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).

(6) 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" x

30” Belt / 5” Disc Sander, Grizzly Industrial, Inc., Springfield, MO) to sand the resin (Figure 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., Worcester, MA) to completely smooth all the sides of the resin. The final sanding with P3000 sand paper ensures 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.

(7) Buffing and polishing. We used a buffer (Central Machinery® 6” Buffer, 1.6amp, 3450RPM, Camarillo, CA) with white compound buffer sticks (Woodstock, 1lb 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) (Figure 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.

3.4 Results and Discussion

This protocol of resin-encapsulation results in professional-looking products (Figure 2A). Using optically-clear resin and carefully buffing all sides results in a safe-to-handle specimen that can be viewed from 6 angles, including under a dissecting scope (Figure 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 for 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 get started with 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 them 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 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), 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. and *Loxosceles* sp.) 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

mosquitos, 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 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.

Product	Cost
Resin purchased from TAP Plastics online Clear-Lite Casting, 1-gal	$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**

Table 3. Cost summary for primary equipment and supplies needed to replicate clear resin-casting protocol outlined here. Cost of shipping not included (Bejcek et al., 2018).

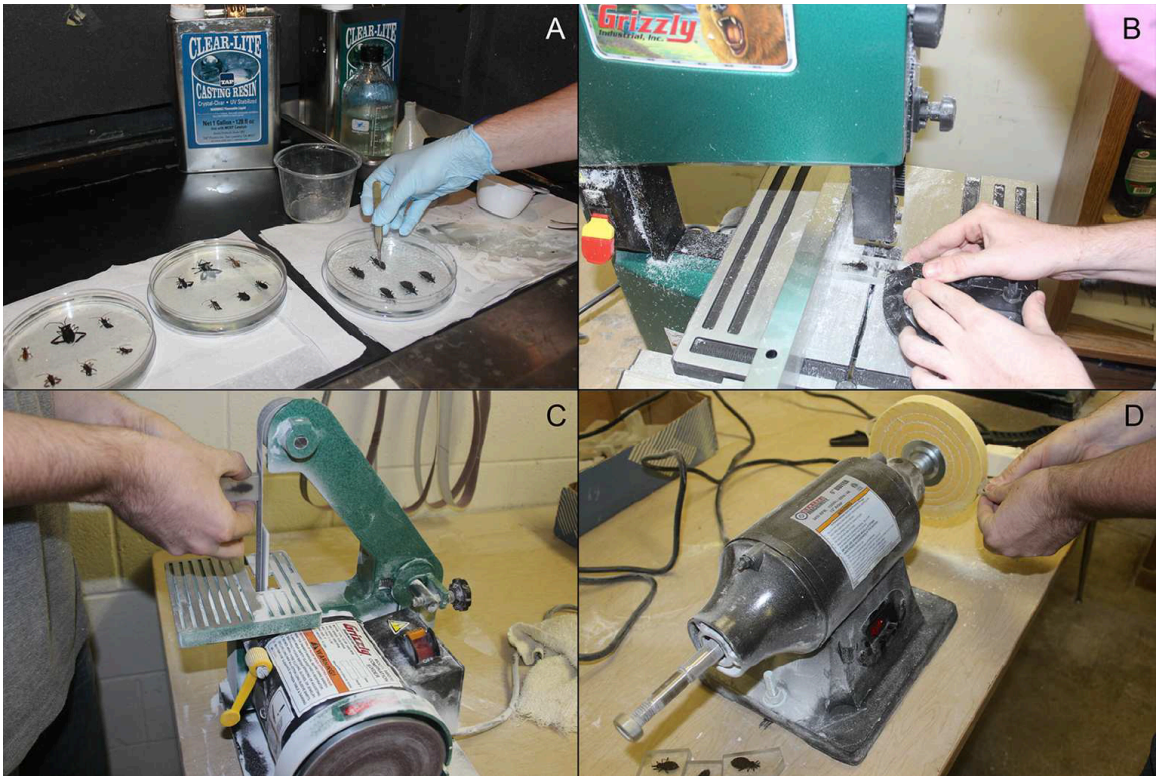

Figure 9. Clear-casting resin production for arthropods showing resin setting in fume hood (A), cutting resin into blocks using band saw (B), sanding resin blocks using belt sander (C), and polishing resin using buffing wheel (D) (Bejcek et al., 2018).

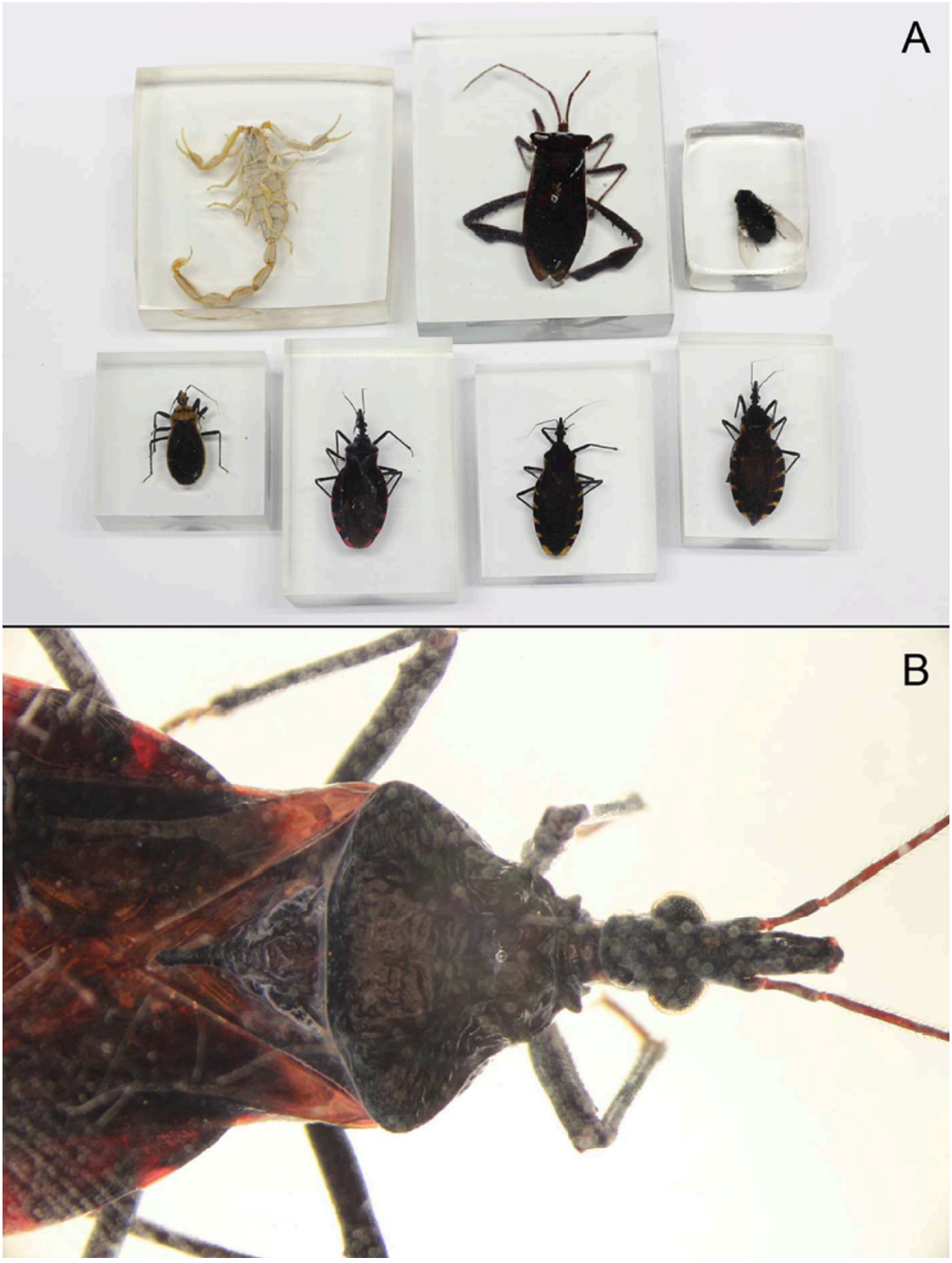

Figure 10. Finished resin blocks containing an assortment of different arthropods (A) and an image of *Triatoma gerstaeckeri* in resin through a dissecting microscope, showing the clarity of anatomic features even through the resin (B) (Bejcek et al., 2018).

CHAPTER IV

CONCLUSION

As we know today, there are over 8 hundred different zoonotic diseases that can infect animals and humans alike, and providing steps to prevent these must include rigorous education campaigns on transmission and disease prevention (Woolhouse et al. 2005; Taylor et al. 2001). For any vector-borne disease, identification of vector species is a key component to such programs. Many other vector-borne diseases have proven this importance by providing identification, localities, disease burden, and many other useful information that help avoid unnecessary anxiety and worry for concerned individuals, such as the tickapp and TickEncounter provided by Texas A&M University and the University of Rhode Island (http://www.tickencounter.org/tick_identification) (http://tickapp.tamu.edu). There is currently little medical and veterinary awareness for Chagas disease in the United States, and carefully-designed outreach tools will be useful in heightening awareness and protecting public health. As for now education and awareness is the primary goal in preventing and treating millions of humans and animals throughout the Americas.

4.1 Innovation

With millions of humans and animals infected with the *T. cruzi* parasite, and millions more at risk, it is imperative to develop means of prevention and treatments for this disease. Providing informative information pertaining to the appropriate identification of kissing bug vectors of *T. cruzi*, and their differentiation from other

commonly observed insects, can allow resources to most efficiently be targeted toward high-risk populations. Access by professionals and the public to resin embedded specimens will aid in prevention. By incorporating these novel educational and outreach initiatives into an established citizen science program, I expect the result will be an informed and empowered public and medical community that with an enhanced ability to combat this neglected tropical disease.

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