

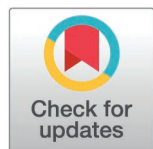
RESEARCH ARTICLE

# Advancing the design of the kissing bug kill trap for surveillance of triatomines

Yuexun Tian<sup>1</sup>, Nadia A. Fernández-Santos<sup>1,2</sup>, Jose G. Juarez<sup>3</sup>, Henry Esquivel<sup>4</sup>, Andrea M. Moller-Vasquez<sup>5</sup>, María Granados-Presa<sup>5</sup>, Adriana Echeverría<sup>5</sup>, Pamela Pennington<sup>5</sup>, Alejandra Zamora-Jerez<sup>5,6</sup>, Juan P. Fimbres-Macias<sup>3</sup>, Walter Roachell<sup>7</sup>, Paul A. Lenhart<sup>7</sup>, Theresa Casey<sup>8</sup>, Molly E. Keck<sup>9</sup>, Carolyn L. Hodo<sup>10</sup>, Christopher H. Downs<sup>1,11</sup>, Sarah C. Sittenauer<sup>1</sup>, Claire C. Nevins<sup>1</sup>, Sujata Balasubramanian<sup>3</sup>, Carlos Angulo<sup>12</sup>, Carlos Palacios-Cardiel<sup>12</sup>, Ramon Gaxiola-Robles<sup>12</sup>, Tania Zenteno-Savín<sup>12</sup>, Sarah A. Hamer<sup>3</sup>, John H. Borden<sup>13</sup>, Michael G. Banfield<sup>11</sup>, Norma Padilla<sup>5</sup>, Gabriel L. Hamer<sup>1\*</sup>

**1** Department of Entomology, Texas A&M AgriLife Research, College Station, Texas, United States of America, **2** Instituto Politécnico Nacional, Centro de Biotecnología Genómica, Reynosa, Mexico, **3** Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, Texas, United States of America, **4** Ministerio de Salud, Departamento de Epidemiología y Control de Vectores, Jutiapa, Guatemala, **5** Centro de Estudios en Salud, Universidad del Valle de Guatemala, Guatemala City, Guatemala, **6** Centro de Estudios en Biotecnología, Universidad del Valle de Guatemala, Guatemala City, Guatemala, **7** United States of America Army Public Health Command West, JBSA-Fort Sam Houston, San Antonio, Texas, United States of America, **8** Lackland Trainee Health Squadron, JBSA-Lackland, San Antonio, Texas, United States of America, **9** Texas A&M AgriLife Extension, San Antonio, Texas, United States of America, **10** Michale E. Keeling Center for Comparative Medicine and Research, The University of Texas MD Anderson Cancer Center, Bastrop, Texas, United States of America, **11** BanfieldBio, Inc., Woodinville, Washington, United States of America, **12** Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Instituto Politécnico Nacional 195, Playa Palo de Santa Rita Sur, La Paz, Baja California Sur, México, **13** JHB Consulting, Burnaby, BC, Canada

\* [gabe.hamer@ag.tamu.edu](mailto:gabe.hamer@ag.tamu.edu)



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**Data availability statement:** The data used for the analyses in this study is available from the supplementary file. Sequencing data from the bloodmeal metabarcoding work along with sample and code information for analysis has

## Abstract

Standardized surveillance and control of kissing bugs (Hemiptera: Reduviidae: Triatominae), the insect vectors of the Chagas disease parasite, *Trypanosoma cruzi*, which causes Chagas disease, remains difficult. The Kissing Bug Kill Trap consists of solar powered LED lights mounted over a column of black funnels. It operates autonomously to capture, kill and preserve adult triatomines. We conducted experiments from 2022–2024 testing potential ways to improve trap performance, ease of deployment, and minimize cost. Thirteen prototypes evaluated in Texas, Guatemala, and Mexico captured 1,531 triatomines. In 2022–2023 we selected a six-funnel trap suspended from a single support pole with an angle bracket, and with four LED lights and a solar panel mounted above the rain-guard, as a reference trap. In 2023, traps with smaller funnels, blue funnels, and blue lights were inferior to the reference trap based on high by-catch of other arthropods and/or fewer triatomines caught per day. In 2024, traps with more or fewer than six funnels or with LED lights mounted on or below the rain guard did not outperform the reference trap. The experiments added five new triatomine species to the four already known to be caught by the Kissing

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**Competing interests:** I have read the journal's policy and the authors of this manuscript have the following competing interests: We declare that the prototype trap developed during this study is associated with a patent application filed on behalf of GLH, MGB, and JHB and assigned to our respective institutions with an assignment to the US government.

Bug Kill Trap and revealed differences and similarities in phenology of dispersal flights of *Triatoma gerstaeckeri* over a three-year period in Texas. The reference trap was selected as the pre-commercial prototype, based on its suitability for triatomine surveillance and potential for reducing the risk of *T. cruzi* infection by intercepting dispersing adult triatomines before they reach human habitats.

## Author summary

As vectors of *Trypanosoma cruzi*, the pathogen causing Chagas disease, triatomine bugs remain difficult to monitor and control due to the lack of standardized tools. In this study, we advanced the Kissing Bug Kill Trap which consists of multiple black funnels and a solar-powered LED light source for triatomine surveillance and potential mass trapping. Over three years of evaluation, we compared variations in funnel size, color, and number, as well as LED light size, color, and placement. Trap performance was assessed by comparing the number of triatomines captured per day and per 1,000 non-target arthropods. The trap design incorporating six large black funnels and four LED panels mounted above the rain guard consistently performed best across multiple regions and countries. A total of 1,531 triatomines representing six species were captured during the study, demonstrating the effectiveness of this trap for targeting multiple triatomine species across diverse geographic areas. Overall, our results indicate that the Kissing Bug Kill Trap has strong potential as a standardized tool for triatomine surveillance and population control due to its high efficiency, low maintenance requirements, and durability.

## Introduction

Triatomines (Hemiptera: Reduviidae: Triatominae) are the insect vectors of *Trypanosoma cruzi*, the agent of Chagas disease throughout the Americas. Surveillance techniques to identify and quantify triatomine populations remain challenging, and the availability of surveillance tools lags behind that for other arthropod vectors such as mosquitoes and ticks. Although pesticides have been used as irritants to flush out triatomines from crevices in homes for surveillance and detection purposes [1], the high cost of manual collections poses a challenge for long-term and large-scale monitoring. Various traps have been developed to capture triatomines for different settings. For example, sticky traps can act as a physical barriers, preventing triatomines from entering chicken coops or residences where they may establish populations [2]. Lorenzo, Reisenman [3] reported a capture of 126 triatomines over five consecutive nights at two chicken-coops using yeast-baited traps, which was significantly more efficient than unbaited traps. In addition to yeast, live mice have also been used as bait, showing high efficiency in nidicolous and domiciliary settings [4,5].

While the above traps target crawling triatomines, several light-based traps have been developed to capture dispersing adult triatomines. These traps capitalize on

the well-established attraction of triatomines to lights [6,7]. In 1964, three black lights hung against a white wall attracted a total of 398 *Triatoma protracta* [8]. Other light trap designs, such as a cross panel trap with diode lights, a cross-vane trap with UV lights, and a white cloth with fluorescent lights, have proven effective in capturing dispersing triatomines under different environmental conditions [2,6,8–10]. Despite their effectiveness, these traps require considerable time to set up and some require manual monitoring, limiting their uses for routine surveillance or large-scale triatomine control. Therefore, there is a need to develop a standardized trap that is easy to deploy, functions autonomously for long periods, and requires minimal maintenance.

Hamer, Fimbres-Macias [11] described the development of the Kissing Bug Kill Trap for collection, killing, and preservation of adult triatomines. The trap is a modified multiple-funnel trap [12] forming a vertical silhouette with an attractive LED light source powered by a solar panel mounted on top of the funnel column. The trap functions autonomously by automatically turning on the LEDs each night using a photodiode and captures attracted arthropods in a collection cup containing propylene glycol as a non-toxic preservative attached beneath the lowest funnel. This trap intercepts flying adult triatomines but not immature stages which lack wings. The trap can be checked at different intervals, depending on operational objectives. Over a three-year development period (2019–2021), Hamer, Fimbres-Macias [11] tested several trap prototypes, captured four triatomine species, and used the traps to evaluate flight dispersal phenology. The objectives of the current study were to evaluate trap performance for capturing triatomines of multiple species in different geographic regions, decrease non-target bycatch, and improve the trap design for commercialization. Experiments conducted in the USA, Mexico, and Guatemala from 2022 to 2024 tested different lengths of the vertical funnel column and different types and positions of LED light stimuli. As triatomines are reportedly attracted to light in the blue (430–555 nm) wavelengths [13–15], we also compared blue and black funnel columns and blue and white LED lights.

## Materials and methods

Experiments were conducted in Texas, USA, Jutiapa, Guatemala, and Baja California Sur, Mexico (Figs 1 and 2). Texas study locations included private properties, public areas, and military training areas in the Lower Rio Grande Valley, San Antonio, Bastrop, and College Station. All the locations were selected based on documented triatomine presence, landscape variability, and availability of collaborators who could manage weekly trap visits. Experiments were set up as randomized complete blocks, with each location considered a replicate across all regions.

The major type of trap used was the Multitrap (Synergy Semiochemicals Corp., Delta, BC, Canada) (Fig 3), a slightly modified Lindgren Funnel Trap, which was designed to mimic a tree trunk and is especially effective for forestry pests. It was modified into the Kissing Bug Kill Trap by adding a visual LED lure [11] and was tested with various modifications of the LED light stimulus and length of the funnel column (Table 1). The LED lights in all traps were controlled by a photoswitch providing illumination throughout the scotophase. All collection cups contained propylene glycol (Bluewater Chemgroup, Fort Wayne, IN, USA), a preservative which can be re-used when checking and resetting traps. Traps within each location were spaced at least 50 m apart and were checked every 1–2 weeks. Collected trap contents were transferred to containers with 70% ethanol and transported to a laboratory. Triatomines were separated from by-catch and identified to species [16]. Other arthropods (by-catch) were identified to order and counted (S1 Fig).

## 2022: Evaluation of light sources

In 2022, three types of Multitraps were evaluated (Fig 3 and Table 1) for the number of light sources and size of the solar panel (TRAP1 versus TRAP2 and TRAP3) and length (TRAP1 and TRAP2 versus TRAP3). TRAP1 and TRAP2 were deployed at one location in San Antonio and three private residences in south Texas, while TRAP1 and TRAP3 were deployed in another location in San Antonio, resulting in five replicates for TRAP1 (two in San Antonio, three in south Texas), four for TRAP2 (two in San Antonio and two in south Texas, Fig 3), and one for TRAP3. Within each location, suitable trap sites with no shade and good visibility were identified and then the trap type was randomly assigned to them.



**Fig 1. Map of the locations of the traps in Texas, USA, Mexico, and Guatemala during 2022-2024.** BRGVSP: Bentsen-Rio Grande Valley State Park; ELGSP: Estero Llano Grande State Park; RDLPSP: Resaca De La Palma State Park; PR: private residence. Administrative boundaries were obtained from the Natural Earth database (<https://www.naturalearthdata.com>) using the R packages *naturalearth* and *naturalearthdata*.

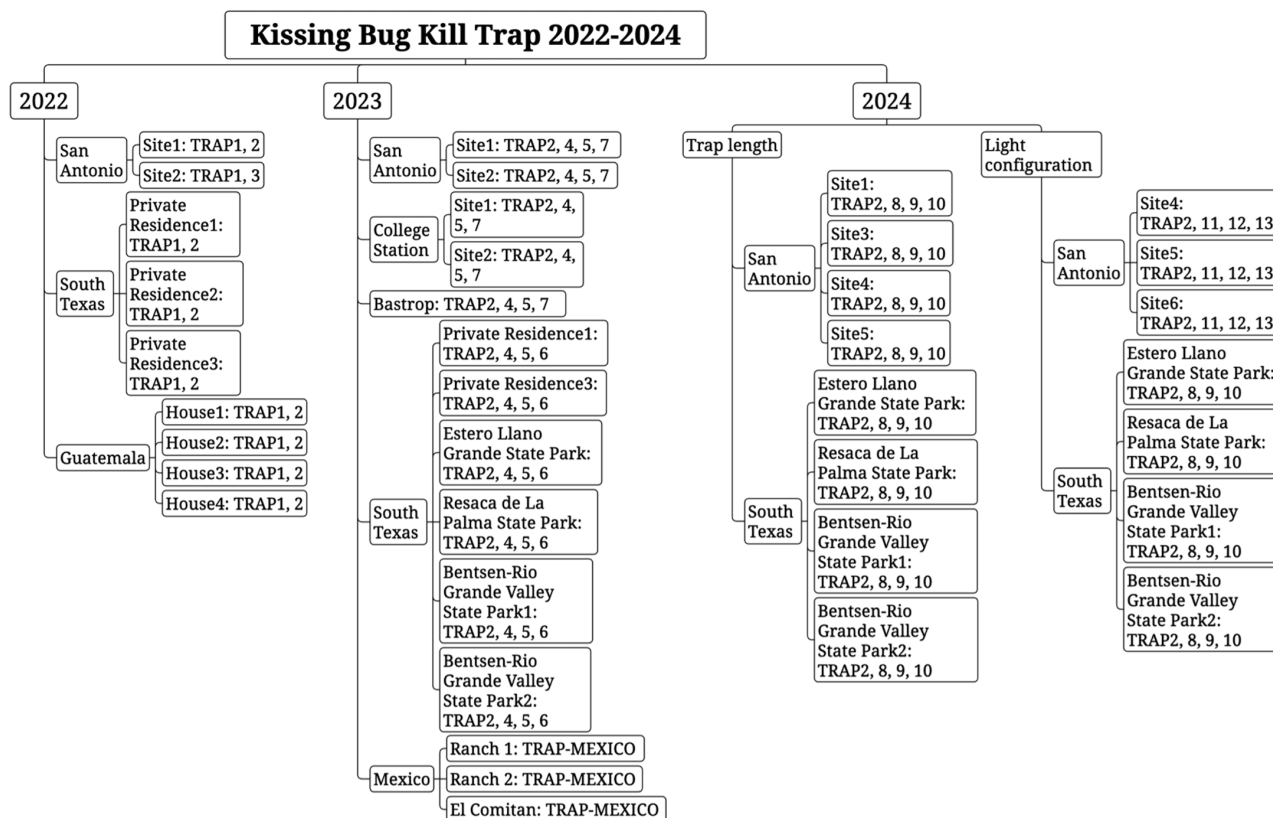
<https://doi.org/10.1371/journal.pntd.0014005.g001>

The traps were installed using two T-posts with different numbers of metal bars supporting the lights above the trap (four bars for large lights and one for small lights) [11]. Traps were monitored from April to October 2022 in San Antonio and April 2022 to April 2023 in South Texas.

In Guatemala, eight traps (four TRAP1 and four TRAP2) were deployed on July 7, 2022, in two communities in Comapa, Jutiapa, selected based on previous presence of *T. dimidiata* at low density in households [17]. The traps in Guatemala had the same funnels and lights as Texas but we sourced local materials (1.9-cm rebar and support brackets) to install the traps (Fig 3). In each community, two houses were selected based on field access, location near to an open field and safety. In each, we deployed one trap of each type within 10 m of each other. Traps were checked weekly from July 2022 to May 2024. Two manual searches for triatomines using the one-person-hour methodology at the households were conducted in May 2023 and May 2024 [18].

### 2023: Color of funnels and light source

TRAP2A was modified from TRAP2 so that the solar panel and LED lights are supported by a single angle bracket and the trap is hung from a bracket attached to a single supporting post; this trap was used as a basis for comparison with the Lindgren multiple-funnel trap modified as in Table 1. In 2023, we focused on the evaluation of funnel size and number as well as light color to evaluate the potential to increase efficacy. Because the Lindgren trap has smaller funnels than the Multitrap, eight funnels were needed in TRAP4 to achieve the same column length as TRAP2 with six funnels. TRAP5 was similar to TRAP4, but with only four funnels. TRAP6 was also similar to TRAP4, but the funnels were manufactured from blue plastic containing



**Fig 2.** Diagram of the evaluation locations of Kissing Bug Kill Trap in Texas, Guatemala, and Mexico with different trap prototypes (Table 1) deployed from 2022-2024.

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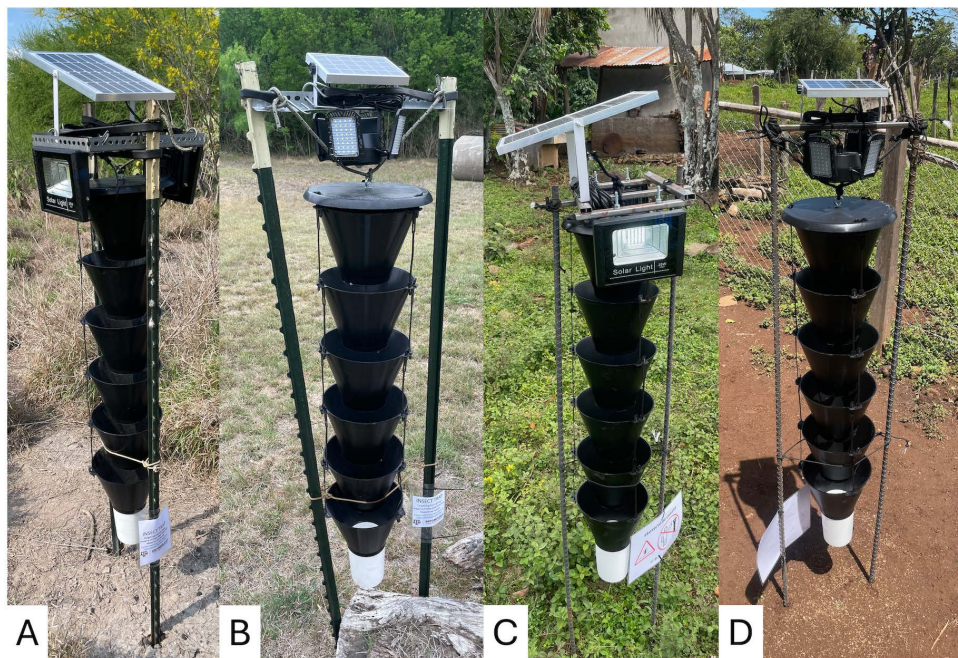
a fluorescent pigment instead of black plastic. TRAP2A, TRAP4, TRAP5, and TRAP6 used the same small 4-panel LED lights. TRAP7 was similar to TRAP6, except that the white LED lights at the top of the trap were replaced with three blue-light LED strips (connected to a 6-volt battery) running vertically down the length of the trap. Suspension of the traps was simplified by using a single T-post with a metal shelving bracket at the top to hold the entire trap (Fig 4).

In central Texas, TRAP2A, TRAP4, TRAP5, and TRAP7, were randomly deployed within two locations in each of San Antonio (eight traps) and College Station (eight traps) and one in Bastrop (four traps), while TRAP2A, TRAP4, TRAP5, and TRAP6 were deployed in each of six locations in south Texas: two private residences (four traps per private residence) and four locations in three state parks [Bentsen-Rio Grande Valley State Park (eight traps), Estero Llano Grande State Park (four traps), Resaca de la Palma State Park (four traps) (Texas Parks and Wildlife State Park Scientific Study Permit No. 26–23)] (Fig 4).

In addition to the five prototypes, another two Lindgren traps of the original design (TRAP-MEXICO, Fig 4 and Table 1) were deployed at three locations in Baja California Sur, Mexico. One trap was deployed at Rancho la Huerta on June 20, 2023, taken down on August 22, 2023, and re-deployed at Rancho José Melero on September 13, 2023. One trap was deployed at El Comitán (CIBNOR weather station) on June 22, 2023. Both traps were removed on December 1, 2023, and redeployed at El Comitán from September 1 to November 15, 2024. The traps were checked every 1–4 weeks during these periods.

## 2024: Pre-commercial configurations

Experiments with modified Multitraps in 2024 focused on evaluation of trap length and position of the LED lights for compatibility with commercialization. In the first evaluation, TRAP2A with six funnels and small 4-panel LED lights



**Fig 3. Kissing Bug Kill Trap prototypes evaluated in 2022.** A) 6-funnel Multitrap trap with two large light panels deployed in Texas (TRAP1); B) 6-funnel Multitrap trap with 4 small light panels deployed in Texas (TRAP2), C) TRAP1 prototype deployed in Guatemala, D) TRAP2 prototype deployed in Guatemala.

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mounted above the rain guard was compared with identical traps with three funnels (TRAP8), nine funnels (TRAP9), and twelve funnels (TRAP10) (Table 1 and Fig 5). Traps were randomly deployed in each of the four locations in south Texas (four traps/location) and the San Antonio area (four traps/location), resulting in eight replicates for each trap length. In the second evaluation, TRAP2A was compared with traps bearing 4-panel LED lights around the perimeter of the rain guard (TRAP11), 4-panel LED lights below the rain guard (TRAP12), and 5-panel LED lights below the rain guard (TRAP13) (Fig 5). Traps were deployed at three locations in south Texas (three traps/location) and San Antonio area (three traps/location), resulting in six replicates for each light position (Fig 2).

### Sample processing methods

For traps from the Texas locations with a large number of insects collected, two methods were used to estimate by-catch. The dry weight method was used to process all TRAP7 collections in 2023. After the removal of all triatomines, the remaining trap content was drained, spread evenly in plastic Petri dishes (13.5 cm diameter, 1.8 cm depth), and dried in a fume hood at room temperature for  $\geq 48$  h. The desiccated arthropods were then weighed in a new petri dish, and approximately 1/8 of the content was removed, weighed again, and all specimens in the 1/8 content were identified to order. The total number of specimens in each order was estimated based on the equation:

$$\text{Estimated total} = \text{Number in subsample} / \frac{\text{subsample weight}}{\text{total content weight}}$$

The floating method was used for the 2024 collections to estimate numbers of small arthropods as the small arthropods tend to stick together and were difficult to separate. A round paper of the same 13.5-cm-diameter as a Petri dish was prepared by drawing four lines that equally divided the paper into eight pie pieces (S2 Fig). This paper was placed under

**Table 1. Descriptions of the Kissing Bug Kill Trap in various Multitrap and Lindgren trap modifications tested for capability to capture triatomines in 2022–2024. Modifications are described in trap length, funnel number, size and color, and LED light-solar panel configurations.**

| Trap prototype | Description                                                                                                                                                                                                                                                                                       |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2022           |                                                                                                                                                                                                                                                                                                   |
| TRAP1          | Multitrap (Synergy Semiochemicals Corp.) with 6 large black funnels (16.5 cm high, 23 cm top diameter, 11.8 cm bottom diameter) with 2 large 5V panels of LED lights above the rain guard and a 35 x 24 cm solar panel                                                                            |
| TRAP2          | Multitrap with 6 large black funnels with 4 small 6.5V panels of LED lights and a 17.5 x 12 cm solar panel mounted above the rain guard on a metal bar between two supporting posts                                                                                                               |
| TRAP3          | Multitrap with 4 large black funnels with 4 small 6.5 V panels of LED lights above the rain guard and a 17.5 x 12 cm solar panel                                                                                                                                                                  |
| 2023           |                                                                                                                                                                                                                                                                                                   |
| TRAP2A         | TRAP2 Multitrap with 6 large black funnels with 4 small panels of LED lights above the rain guard, modified to employ a single supporting post with an angle bracket holding the lighting assembly and suspending the trap                                                                        |
| TRAP4          | Lindgren trap (BanfieldBio Inc.) with 8 small black funnels (10.5 cm high, 20 cm top diameter, 6 cm bottom diameter) modified so that the bottoms of all but the lowest funnel are at the same level as the top of the funnels below them, with 4 small panels of LED lights above the rain guard |
| TRAP5          | Lindgren trap with 4 small black funnels modified so that the bottoms of all but the lowest funnel are at the same level as the top of the funnels below them, with 4 small panels of LED lights above the rain guard                                                                             |
| TRAP6          | Lindgren trap with 8 small blue funnels modified so that the bottoms of all but the lowest funnel are at the same level as the top of the funnels below them, with 4 small panels of LED lights above the rain guard                                                                              |
| TRAP7          | Lindgren trap with 8 small blue funnels modified so that the bottoms of all but the lowest funnel are at the same level as the top of the funnels below them, with 3 blue LED light strips start from the first funnel to the last funnel                                                         |
| TRAP-MEXICO    | Lindgren trap with 11 small black funnels with the bottoms of all but the lowest funnel nested within the top portion of the next lowest funnel (original configuration), with 4 small panels of LED lights above the rain guard                                                                  |
| 2024           |                                                                                                                                                                                                                                                                                                   |
| TRAP2A         | Multitrap with 6 large black funnels with 4 small panels of LED lights above the rain guard                                                                                                                                                                                                       |
| TRAP8          | Multitrap with 3 large black funnels with 4 small panels of LED lights above the rain guard                                                                                                                                                                                                       |
| TRAP9          | Multitrap with 9 large black funnels with 4 small panels of LED lights above the rain guard                                                                                                                                                                                                       |
| TRAP10         | Multitrap with 12 large black funnels with 4 small panels of LED lights above the rain guard                                                                                                                                                                                                      |
| TRAP11         | Multitrap with 6 large black funnels with 4 small panels of LED lights on the perimeter of the rain guard                                                                                                                                                                                         |
| TRAP12         | Multitrap with 6 large black funnels with 4 small panels of LED lights under the rain guard                                                                                                                                                                                                       |
| TRAP13         | Multitrap with 6 large black funnels with 5 small panels of LED lights under the rain guard                                                                                                                                                                                                       |

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a plastic Petri dish with the trap contents floating inside the Petri dish. The arthropods that were floating in the water were identified and counted individually. The remaining small arthropods that sank to the bottom were estimated by counting and identifying them to order in three randomly selected pie sections. The total number of specimens in each order was estimated based on the equation:

$$\text{Estimated total} = \text{Number in the subsample} / 3 * 8$$

Two triatomines collected from traps deployed in Guatemala were processed with our lab's established bloodmeal metabarcoding protocol [18] to identify vertebrates fed on prior to capture. These two individuals were also tested for *T. cruzi* using PCR targeting a 166 bp region of repetitive microsatellite nuclear DNA.

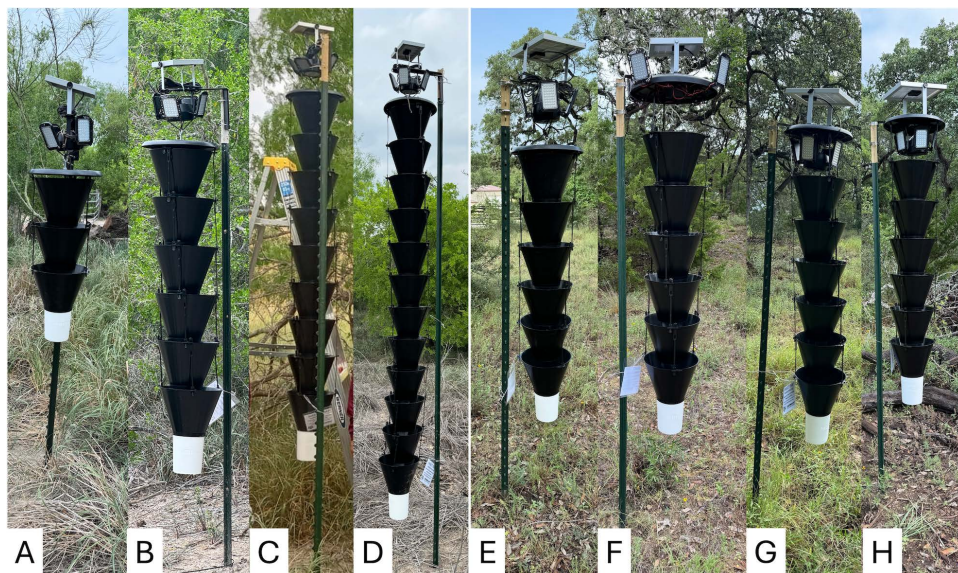
## Statistics

Data for each year were analyzed separately. A generalized linear mixed model (GLMM) was used to compare triatomines caught per day and triatomines per 1000 other arthropods among trap prototypes, with the prototypes as variables and the location as random effects. Tweedie distributions were applied because the data were right-skewed and zero-inflated, a common characteristic in ecological count data. Due to loss during transport, some of the captured triatomines were not identified to species in the lab



**Fig 4. Five Kissing Bug Kill Trap prototypes evaluated in Texas in 2023.** A) TRAP2A, Multitrap with 6 funnels and 4-panel lights; B) Lindgren black funnel trap with 8 funnels and 4-panel lights (TRAP4); C) Lindgren black funnel with 4 funnels and 4-panel light (TRAP5); D) Lindgren blue funnel trap with 8 funnels and 4-panel light; E) Lindgren blue funnel trap with 8 funnels and blue LED light strips (TRAP7); F) Lindgren black funnel nested together with 11 funnels and small LED (TRAP-MEXICO).

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**Fig 5. Kissing Bug Kill Traps deployed in Texas in 2024.** Group one compares Multitraps with A) TRAP8, 3 funnels, B) TRAP2A, 6 funnels, C) TRAP9, 9 funnels, and D) TRAP10, 12 funnels, and Group 2 compares Multitraps with E) TRAP2A, 4 LED lights above the rainguard, F) TRAP11, 4 LED lights on the perimeter of rainguard, G) TRAP12, 4 LED lights under the rainguard, and H) TRAP13 5 LED lights under the rainguard.

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and were not included in the species-based analyses. The identified triatomine numbers were also analyzed using a GLMM, with a negative binomial distribution, with species and sex as the explanatory variables and years as random effects. A pairwise comparison evaluated the effect of sex on triatomine number within each species. All analyses were conducted using R [19]. Data for which we could not confirm accuracy due to unclear labeling and records were excluded from the analyses.

## Results

During the 3-year evaluation period, the Kissing Bug Kill Trap prototypes captured 1,531 triatomines with an estimated 604,432 non-target arthropods (S1 Table).

### 2022: Evaluation of light sources

In 2022 in Texas, 197 triatomines were captured by the three trap prototypes (five TRAP1, four TRAP2, and one TRAP3) (Table 2). Due to 47 lost specimens during relocation from our field laboratory to the main laboratory, only 150 triatomines were identified to species: 49 female and 98 male *T. gerstaeckeri* and 1 female and 2 male *T. sanguisuga*. Mean triatomines caught per day did not differ significantly among the three trap prototypes with Traps 1 and 3 being the highest and the lowest, respectively. However, the mean triatomines per 1,000 other arthropods for TRAP3 (11.29) was significantly higher than for TRAP1 (10.61). Although TRAP2 (6.55 triatomines), with six funnels and small LED lights, captured fewer triatomines than the prototype with large LED lights and 6 funnels (TRAP1), the difference was not significant. These results were due to the varied number of each trap evaluated (four TRAP2 and one TRAP3), resulting in different variances. Moreover, TRAP2 was light and only required one support metal bar while TRAP1 with large LED lights was heavier and required two metal bars to mount the light and solar panel securely to the stakes.

In Comapa, Guatemala, 13 triatomines, all *T. dimidiata*, were captured from the eight traps during 2022–2024 (Table 3). Traditional manual searches of four homes found no triatomines. Two *T. dimidiata* (collected on October 1 and November 27, 2022) were processed for bloodmeal metabarcoding [20]. Only one (female) yielded a PCR amplicon of the vertebrate 12S rRNA gene followed by amplicon deep sequencing, which matched *Canis lupus familiaris* (domestic dog) and *Gallus gallus* (chicken). This same female (TD22CompaKB1) was also infected with *T. cruzi* (untypable).

### 2023: Color of funnels and light source

In 2023 in Texas, 44 traps of 5 prototypes caught 492 triatomines with 460 identified to species due to specimen lost during transportation, including 83 female and 347 male *T. gerstaeckeri*, 7 female and 9 male *Paratriatoma lectionaria*,

**Table 2. Total numbers of triatomines caught and operational days for each trap prototype in Texas in 2022 with means for trap performance and by-catch ratio calculated. A generalized mixed linear model was used to compare trap performance and by-catch ratio with TRAP1 as reference. Asterisk indicates significant difference from TRAP1 ( $p < 0.05$ ). Dash indicates that the trap was not evaluated at the location. The two San Antonio locations are at the Joint Base San Antonio (JBSA)-Lackland Chapman Training Annex.**

|                                             | TRAP1  | TRAP2 | TRAP3   |
|---------------------------------------------|--------|-------|---------|
| Number of triatomines                       |        |       |         |
| San Antonio-1                               | 14     | 42    | –       |
| San Antonio-2                               | 65     | –     | 17      |
| Private residence-1(Mission-North)          | 10     | 14    | –       |
| Private residence-2 (Mission-South)         | 20     | 8     | –       |
| Private residence-3 (Mission-South2)        | 6      | 1     | –       |
| Number of operational days                  |        |       |         |
| San Antonio-1                               | 188    | 188   | –       |
| San Antonio-2                               | 188    | –     | 188     |
| Private residence-1                         | 357    | 358   | –       |
| Private residence-2                         | 71     | 71    | –       |
| Private residence-3                         | 279    | 279   | –       |
| Mean triatomines per day                    | 0.150  | 0.095 | 0.090   |
| Mean triatomines per 1,000 other arthropods | 10.613 | 6.553 | 11.288* |

<https://doi.org/10.1371/journal.pntd.0014005.t002>

**Table 3. Total number of triatomines caught and operational days for each trap prototype in Guatemala from 2022-2024 with mean trap performance and by-catch ratio calculated. A generalized mixed linear model disclosed no significant differences in trap performance and by-catch ratio with TRAP1 as reference.**

|                                             | TRAP1 | TRAP2 |
|---------------------------------------------|-------|-------|
| Number of triatomines                       |       |       |
| House 1                                     | 1     | 2     |
| House 2                                     | 1     | 0     |
| House 3                                     | 5     | 1     |
| House 4                                     | 3     | 0     |
| Number of operational days                  |       |       |
| House 1                                     | 660   | 667   |
| House 2                                     | 653   | 660   |
| House 3                                     | 660   | 667   |
| House 4                                     | 667   | 667   |
| Mean triatomines per day                    | 0.004 | 0.001 |
| Mean triatomines per 1,000 other arthropods | 0.181 | 0.066 |

<https://doi.org/10.1371/journal.pntd.0014005.t003>

4 female and 4 male *Hospesneotomae neotomae* [formally known as *Triatoma neotomae* [21]], and 3 female and 3 male *T. sanguisuga* (Table 4). TRAP2A (with 6 large black funnels) had the highest number of triatomines per day, significantly higher than TRAP4 (with 8 small black funnels) and TRAP5 (with 4 small black funnels). It also had significantly more triatomines per 1,000 other arthropods than TRAP7 (with blue funnels and blue light) ( $p \leq 0.01$ ). Meanwhile, TRAPS 5 and 7 had the lowest triatomines per day and per 1,000 other arthropods, respectively.

Four triatomines were captured from the two traps in Mexico. Two *Dipetalogaster maxima* (one female and one male) were captured at Rancho la Huerta on July 21, 2023. One female and one male *Hospesneotomae peninsular* [formerly known as *Triatoma peninsular* [21]] were captured at El Comitán (CIBNOR weather station) on October 18, 2024.

## 2024: Pre-commercial configurations

In 2024 in Texas, 690 triatomines were collected, 333 in the 32 traps with different numbers of funnels and 357 in the 24 traps with different light configurations (Tables 5 and 6). The 626 triatomines identified to species included 175 female and 411 male *T. gerstaeckeri*, 2 female and 4 male *T. indictiva*, 8 female and 8 male *P. lecticulari*, 3 female and 3 male *H. neotomae*, and 2 female and 5 male *T. sanguisuga*, with 5 specimens too damaged to be identified, with 64 specimens lost during transportation.

Traps with different number of funnels (3, 6, 9, 12 funnels) did not have a significant difference in the triatomine numbers while traps with 9 and 3 funnels had the highest and lowest numbers of triatomines per day and per 1,000 other arthropods, respectively. (Table 5). TRAP2A caught significantly more triatomines per day and significantly more triatomines per 1,000 other arthropods than traps with the LED lights on the perimeter of (TRAP11) or below (TRAP12, TRAP13) the rain guard (Table 6). Trap 11 and trap 12 had the lowest triatomines per day and triatomines per 1,000 other arthropods, respectively. Increasing the number of LED lights from four to five in TRAP13 raised the number of triatomines caught per day to a level not significantly different from that in TRAP2A.

## Triatomine species and sex ratios

*Triatoma gerstaeckeri* was caught in significantly higher numbers ( $z = 5.311$ ,  $P < 0.001$ ) compared to all other species (Fig 6), with 94.1% ( $n = 1,156$ ) of all captures. There was no interaction between sex and triatomine species ( $P > 0.1$ ). Pairwise comparisons revealed 2.8x more *T. gerstaeckeri* males than females captured ( $P < 0.01$ ) but there was no

**Table 4. Total number of triatomines caught and operational days for each trap prototype in Texas in 2023 with mean trap performance and by-catch ratio calculated. A generalized mixed linear model was used to compare trap performance and by-catch ratio with TRAP2A trap as a reference. Asterisks indicate significant difference from TRAP2A ( $p \leq 0.05$ ). The two San Antonio locations are at the Joint Base San Antonio (JBSA)-Lackland Chapman Training Annex.**

|                                             | TRAP2A | TRAP4  | TRAP5  | TRAP6 | TRAP7  |
|---------------------------------------------|--------|--------|--------|-------|--------|
| Number of triatomine                        |        |        |        |       |        |
| San Antonio-1                               | 7      | 4      | 5      | —     | 3      |
| San Antonio-2                               | 4      | 4      | 6      | —     | 6      |
| College Station-1                           | 0      | 0      | 0      | —     | 0      |
| College Station-2                           | 0      | 0      | 0      | —     | 0      |
| Bastrop                                     | 3      | 3      | 1      | —     | 1      |
| Estero Llano Grande State Park              | 96     | 10     | 9      | 21    | —      |
| Resaca De La Palma State Park               | 53     | 45     | 6      | 13    | —      |
| Bentsen-Rio Grande Valley State Park -1     | 0      | 6      | 2      | 20    | —      |
| Bentsen-Rio Grande Valley State Park -2     | 85     | 13     | 8      | 12    | —      |
| Private residence-1                         | 3      | 14     | 9      | 19    | —      |
| Private residence-4                         | 1      | 0      | 0      | 0     | —      |
| Number of operational days                  |        |        |        |       |        |
| San Antonio-1                               | 63     | 62     | 49     | —     | 56     |
| San Antonio-2                               | 71     | 61     | 67     | —     | 42     |
| College Station-1                           | 95     | 95     | 95     | —     | 59     |
| College Station-2                           | 108    | 101    | 99     | —     | 81     |
| Bastrop                                     | 129    | 129    | 129    | —     | 129    |
| Estero Llano Grande State Park              | 380    | 367    | 317    | 372   | —      |
| Resaca De La Palma State Park               | 373    | 373    | 334    | 373   | —      |
| Bentsen-Rio Grande Valley State Park -1     | 368    | 368    | 336    | 368   | —      |
| Bentsen-Rio Grande Valley State Park -2     | 372    | 372    | 335    | 372   | —      |
| Private residence-1                         | 368    | 359    | 332    | 368   | —      |
| Private residence-4                         | 369    | 369    | 333    | 349   | —      |
| Mean triatomines per day                    | 0.075  | 0.035* | 0.027* | 0.038 | 0.041  |
| Mean triatomines per 1,000 other arthropods | 5.052  | 3.445  | 3.9102 | 3.723 | 0.072* |

<https://doi.org/10.1371/journal.pntd.0014005.t004>

difference in catch by sex for *T. indictiva* ( $P=0.30$ ), *P. lecticularia* ( $P=0.51$ ), *H. neotomae* ( $P=0.79$ ), and *T. sanguisuga* ( $P=0.48$ ).

## Phenology

Adult flight dispersal phenology for *T. gerstaeckeri* plotted as the numbers of bugs caught per day per trap among the traps for each week of sampling differed between years and regions (Fig 7). Dispersal flights in 2022 had begun in both south Texas and San Antonio when traps were deployed in April, and dispersal activity in both places diminished by the first week of August. The 2023 and 2024 dispersal flights in South Texas both began in the third week of March. The 2023 flight ended in the third week of September, while the 2024 flight extended into October. In both years flight in San Antonio began several weeks later and ended several weeks earlier than in South Texas.

Other triatomine species in Texas were captured in smaller numbers than *T. gerstaeckeri* (S2 Table). *Triatoma sanguisuga* were captured from May to August, *H. neotomae* from April to October, *T. indictiva* in July and August, and *P. lecticularia* from April to September. Thirteen *T. dimidiata* were captured throughout the year in Guatemala, three during the rainy season from May to October and 10 during the dry season from November to April.

**Table 5. Total number of triatomines caught and operational days for trap prototypes with different numbers of funnels in Texas in 2024 with mean trap performance and by-catch ratio calculated. A generalized mixed linear model disclosed no significant differences in trap performance and by-catch ratio with the TRAP2A trap as reference. San Antonio-1 and San Antonio-3 are at the Joint Base San Antonio (JBSA)-Lackland Chapman Training Annex.**

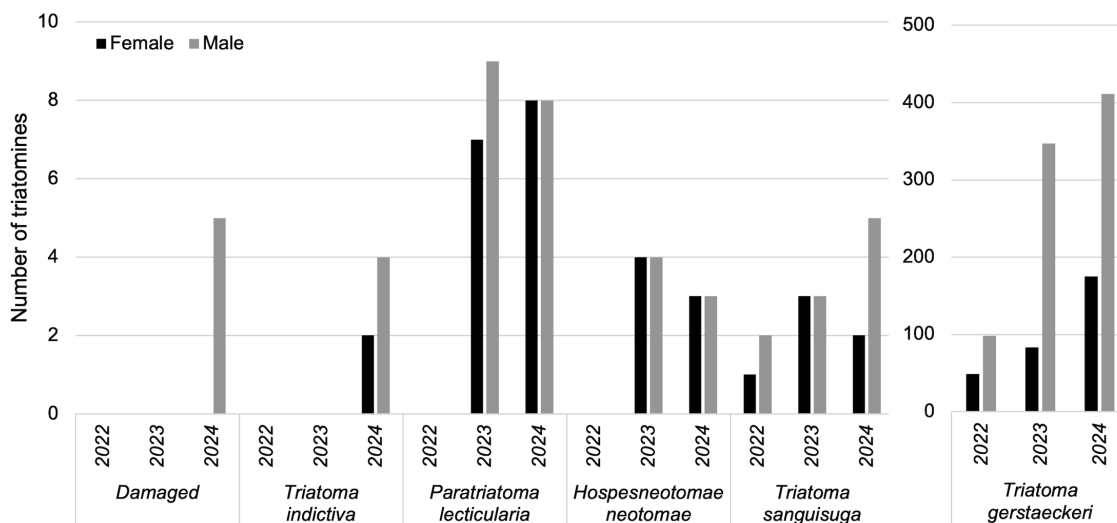
|                                             | TRAP2A | TRAP8 | TRAP9 | TRAP10 |
|---------------------------------------------|--------|-------|-------|--------|
| Number of triatomine                        |        |       |       |        |
| San Antonio-1                               | 1      | 0     | 8     | 3      |
| San Antonio-3                               | 7      | 1     | 0     | 3      |
| San Antonio-4                               | 0      | 1     | 3     | 7      |
| San Antonio-5                               | 1      | 1     | 4     | 1      |
| Resaca De La Palma State Park               | 4      | 4     | 25    | 13     |
| Bentsen-Rio Grande Valley State Park -1     | 8      | 42    | 11    | 17     |
| Bentsen-Rio Grande Valley State Park -2     | 56     | 2     | 24    | 16     |
| Estero Llano Grande State Park              | 12     | 9     | 23    | 26     |
| Number of operational days                  |        |       |       |        |
| San Antonio-1                               | 113    | 114   | 113   | 113    |
| San Antonio-3                               | 107    | 113   | 113   | 110    |
| San Antonio-4                               | 113    | 113   | 113   | 113    |
| San Antonio-5                               | 114    | 114   | 114   | 114    |
| Resaca De La Palma State Park               | 215    | 215   | 215   | 191    |
| Bentsen-Rio Grande Valley State Park -1     | 214    | 214   | 214   | 209    |
| Bentsen-Rio Grande Valley State Park -2     | 214    | 214   | 214   | 214    |
| Estero Llano Grande State Park              | 214    | 214   | 214   | 214    |
| Mean triatomines per day                    | 0.057  | 0.037 | 0.065 | 0.059  |
| Mean triatomines per 1,000 other arthropods | 0.431  | 0.311 | 0.731 | 0.566  |

<https://doi.org/10.1371/journal.pntd.0014005.t005>

**Table 6. Total number of triatomines caught and operational days for trap prototypes with different light configurations in Texas in 2024 with mean trap performance and by-catch ratio calculated. A generalized mixed linear model was used to compare trap performance and by-catch ratio with the TRAP2A trap as reference. Asterisks indicate a significant difference from TRAP2A ( $p \leq 0.05$ ).**

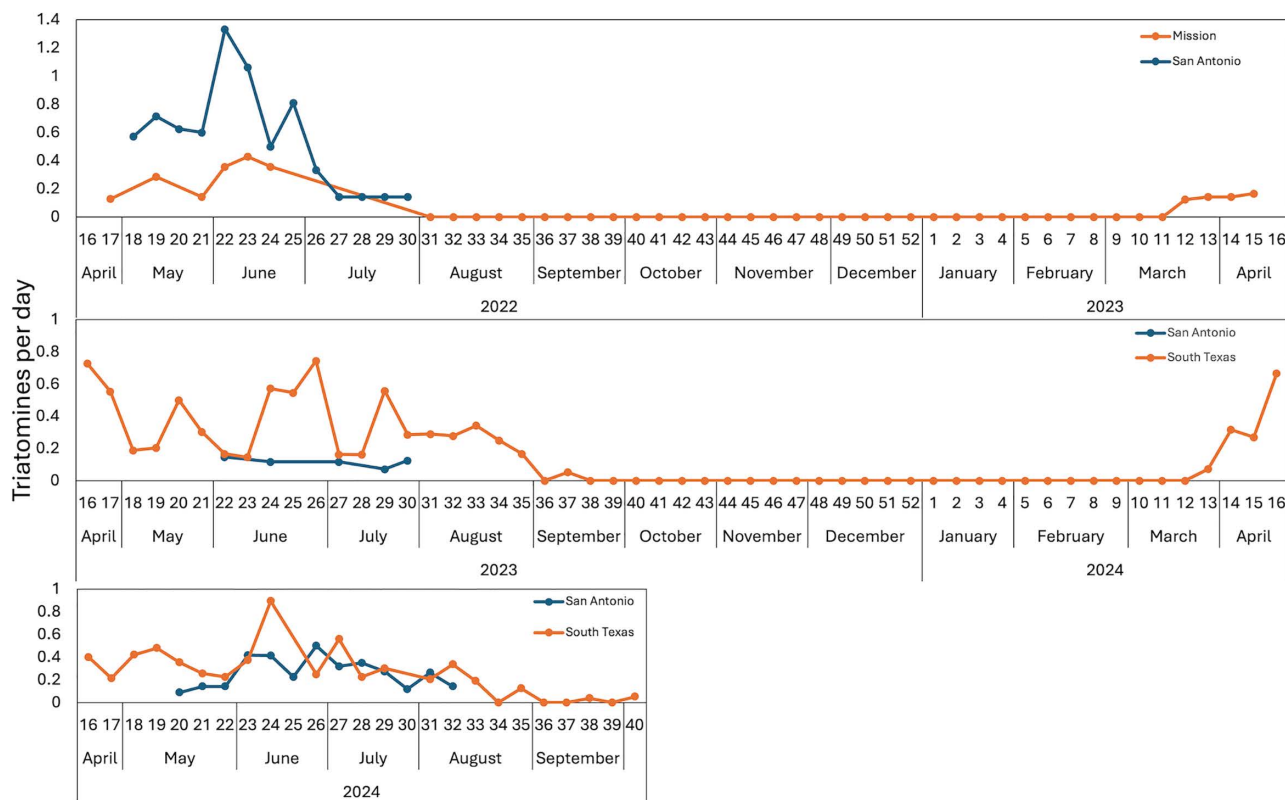
|                                             | TRAP2A | TRAP11 | TRAP12 | TRAP13 |
|---------------------------------------------|--------|--------|--------|--------|
| Number of triatomine                        |        |        |        |        |
| San Antonio-4                               | 4      | 2      | 2      | 4      |
| San Antonio-5                               | 5      | 1      | 4      | 2      |
| San Antonio-6                               | 32     | 16     | 24     | 34     |
| Resaca De La Palma State Park               | 76     | 4      | 20     | 30     |
| Bentsen-Rio Grande Valley State Park        | 10     | 3      | 3      | 11     |
| Estero Llano Grande State Park              | 22     | 6      | 18     | 24     |
| Number of operational days                  |        |        |        |        |
| San Antonio-4                               | 69     | 69     | 69     | 69     |
| San Antonio-5                               | 69     | 69     | 69     | 69     |
| San Antonio-6                               | 72     | 69     | 72     | 69     |
| Resaca De La Palma State Park               | 171    | 171    | 171    | 171    |
| Bentsen-Rio Grande Valley State Park        | 164    | 164    | 164    | 164    |
| Estero Llano Grande State Park              | 164    | 164    | 164    | 164    |
| Mean triatomines per day                    | 0.202  | 0.059* | 0.111* | 0.161  |
| Mean triatomines per 1,000 other arthropods | 0.807  | 0.284* | 0.282* | 0.359* |

<https://doi.org/10.1371/journal.pntd.0014005.t006>



**Fig 6.** Number of each triatomine species and sex collected from the traps from 2022-2024.

<https://doi.org/10.1371/journal.pntd.0014005.g006>



**Fig 7.** Weekly phenology of *Triatoma gerstaeckeri* catch per day per trap in 2022-2024 at south Texas and San Antonio. The trap data for all south Texas locations and all San Antonio locations are averaged.

<https://doi.org/10.1371/journal.pntd.0014005.g007>

## Trap by-catch and failures

The by-catch, measured as triatomines per 1,000 other arthropods, ranged from 0.04 for TRAP7 (blue funnels with blue LED light strips) to 11.29 for TRAP3 (4 black funnels and 4 LED lights) for the 15 trap prototypes that received the full processing of total arthropods (S1 Table). While the TRAP7 blue funnel and blue light trap was only operated for one season in Texas, this trap captured 261,495 individual arthropods, which is 43.3% of all the by-catch of all 15 prototypes for the entire three-year study. The longitudinal pattern in by-catch of other arthropods showed peaks in early June and late August in south Texas, 2022 and in June in San Antonio in 2023 and 2024 (Fig 7).

Over the three-year evaluation period of 15 traps, occasional malfunctions were observed and promptly addressed. For example, connections on one 12-funnel trap in 2024 broke and the trap fell; the trap was repaired to restore functionality. Similarly, malfunctioning lights were either fixed or replaced as soon as detected. At one site in San Antonio (Lackland Chapman Training Annex) in 2024, the solar panel light from one trap was stolen; we replaced the missing light with a backup unit to maintain continuous operation. Collection data obtained from all affected traps during malfunction periods were discarded and excluded from analyses.

## Discussion

Over a period of three years (2022–2024), we captured 1,531 triatomines of eight species in 13 Kissing Bug Kill Trap prototypes in three countries. Among the 13 trap prototypes, the combination of six large black funnels with 4 LED light panels mounted above the rain guard with a single support pole and angle bracket (TRAP 2A) performed best in terms of triatomine caught per day, and per 1,000 other arthropods and overall ease of deployment.

In 2022, TRAP2 with six black funnels, two support posts and four small LED light panels mounted above the rain guard on a bar suspended between the support posts was comparable to the same trap with two large light panels (TRAP1) in triatomines caught per day but was inferior in triatomines caught per 1,000 other arthropods. This inferiority was not found in later experiments. Moreover, the LED lights and solar panel of TRAP2 were lighter than the larger LED lights and solar panel of TRAP1, which required a cumbersome square frame mounted to the two support posts. Because TRAP2 was lighter, cheaper, and easier to install than TRAP1, it was modified by employing a single supporting post with an angle bracket holding the lighting assembly and suspending the trap (called TRAP2A). TRAP2A was selected as a reference treatment for experiments in 2023 and 2024. These experiments upheld its superiority.

In 2023, TRAP2A outperformed all other traps tested regardless of color or length of the funnel column, and in 2024 it outperformed other Multitrap prototypes with either different numbers of funnels or lower LED light positions. Lindgren traps were designed to catch flying beetles [12] and the modifications incorporated into Multitraps improved their efficacy for this use [22]. Unlike the original Lindgren trap [designated as TRAP-MEXICO (Fig 4F)] which had nested funnels, other versions of the Lindgren trap in our experiments had the funnels configured so that the bottom of one funnel was at the same level as the top of the funnel below it (Fig 4B–4E), just as the funnels in TRAP2 and TRAP2A were configured. Therefore, other features of TRAP2 and TRAP2A would have been responsible for their superior performance. These features include wider funnels and apertures, greater funnel height and a steeper slope.

Despite evidence that triatomines are attracted to blue light [13–15], blue Lindgren traps with white LED lights (TRAP6) or blue LED strips (TRAP7) caught no more triatomines than black traps with white LED lights. The blue traps with blue LED strips were highly attractive to other arthropods, resulting in significant reduction in triatomines caught per 1,000 other arthropods. Thus, they are less suitable for catching triatomines but might be useful in ecological studies that investigate taxonomic diversity and biomass of night-flying insects.

Hamer, Fimbres-Macias [11] demonstrated that the Kissing Bug Kill Trap captured four species of triatomines: *T. gerstaeckeri*, *T. sanguisuga*, *H. neotomae* and *T. rubida*. Our study adds five additional species to this list: *P. lecticularia* and *T. indictiva* in the USA, *D. maxima* and *H. peninsularis* in Mexico, and *T. dimidiata* in Guatemala. The triatomine species collected in the USA likely reflect differences in population abundance and distribution across ecotopes. *Triatoma*

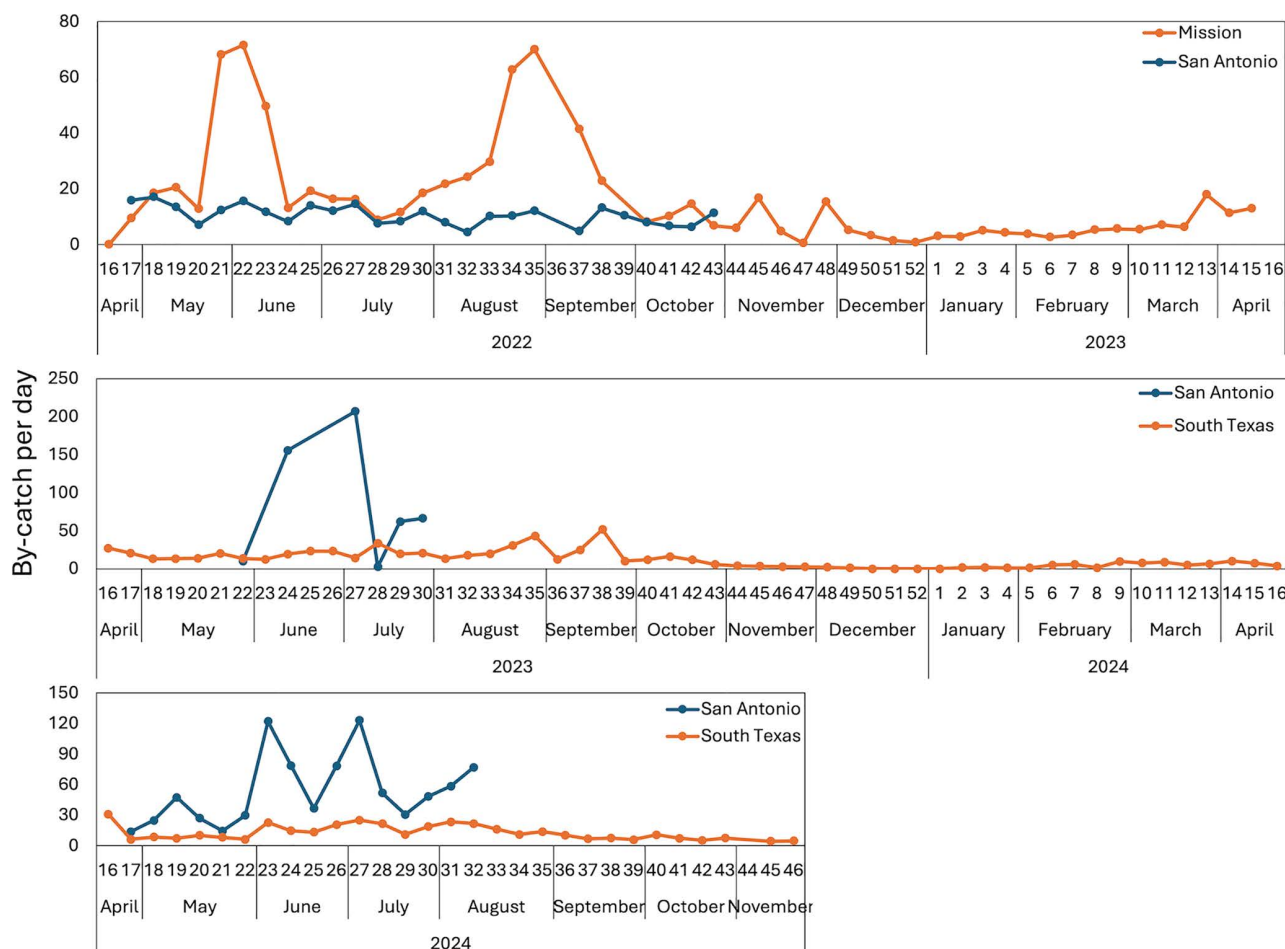
*gerstaeckeri* was the dominant species captured by the traps in central and south Texas, corroborating data collected in a citizen-science study in Texas [23]. The additional triatomine species collected in Texas are known to occur in these regions but at lower abundance [24], demonstrating that relative capture of dispersing adult triatomines of the different species by the Kissing Bug Kill Trap likely reflects local population densities. The low number of *T. dimidiata* in Comapa, Guatemala may be due to low triatomine populations in sylvatic habitats in the region [18] due to anthropogenic disturbance to natural habitat and the subsistence hunting of wild animals resulting in low wildlife occurrence [25]. The eight traps in Guatemala were in continuous operation for over two years with minimal maintenance, indicating their durability and suitability for use in rural areas of Central America. Two manual searches conducted inside the Guatemala households with traps installed outside yielded zero triatomines, suggesting that in addition to serving as a more effective surveillance tool than manual searching, the traps may have intercepted dispersing triatomines before they entered households and established populations. Sampling of other households in these same Comapa, Guatemala communities in 2022 disclosed infestation rates ranging from 17–38% and colonization levels between 9–29% [18].

Prior to development of the Kissing Bug Kill Trap, no standardized surveillance tool has existed to attempt to quantify spatio-temporal heterogeneity of adult triatomine abundance. We have now deployed large numbers of these traps over six consecutive seasons in multiple countries and have begun to capture the species diversity of dispersing adult triatomines as well as variation in adult dispersal phenology. Confirming anecdotal observations in prior years, trap catches in south Texas and San Antonio comprised mainly *T. gerstaeckeri*, with varying magnitudes and peaks (Fig 7). Within a season, the earlier occurrence in traps of *T. gerstaeckeri* in south Texas compared to San Antonio matches our prior observations. Using traps as a standardized surveillance tool will remove observer bias associated with other forms of collection.

While we captured 1,531 triatomines during three years of field experiments, we also captured 604,432 non-target arthropods (S1 Table). While this amount of by-catch sounds large, several interpretive factors need to be considered. Of the total by-catch, 43.3% was collected from the TRAP7 (blue funnel with three blue light stripes) in 2023. Low catches of target insects (only 10 triatomines) coupled with the very high by-catch led to the decision to withdraw this trap from further evaluation. The magnitude of by-catch fluctuated throughout the years in different locations (Fig 8). To evaluate the phenology of triatomines in regions with poor understanding of seasonal changes in dispersal behavior, these traps were set up and run continuously for multiple months or multiple years. This study design therefore results in traps being deployed during periods of active triatomine dispersal and also periods of no triatomine captures when other non-target arthropods continued to be captured. For example, large numbers of non-target arthropods and no triatomines were captured in the traps in August and September, 2022, in south Texas (Figs 7 and 8). Even when studies quantify trap by-catch and present ratios of vectors to non-target captures [26], this is only done during short windows of time and we are un-aware of any arthropod vector trap that is continuously operated for multiple years.

Non-target by-catch in Kissing Bug Kill Traps could have alternative scientific uses, such as in studies of biodiversity, dispersal and phenology of numerous flying and passively dispersing insect species [27]. In addition, by-catch could be used for animal feed, e.g., poultry food for rural villages in developing countries. This award-winning concept of mass capture of flying insects for animal feed was introduced by Cohnstaedt, Lado [28]. It may or may not be necessary to find an alternative for propylene glycol, which is generally regarded as safe, with an LD<sub>50</sub> of 21,000 mg/kg in dogs (Propylene Glycol | C3H8O2 | CID 1030 - PubChem).

It is difficult to compare the by-catch observed with different Kissing Bug Trap prototypes because other vector trapping studies rarely quantify by-catch (but see Hribar [29]). While trap by-catch often makes the removal of vectors time-consuming, that is not the case with triatomines in Kissing Bug Kill Traps. Triatomines are larger than most of the arthropod by-catch (see S1–S2 Figs) and also have a distinctive morphology. Therefore, when processing traps, the triatomines are easily removed as the first quick step and then the very consuming step (for research purposes only) is identifying, sorting, and counting the other arthropods (S1 Fig).



**Fig 8. Weekly number of by-catch per day per trap in 2022–2024 at south Texas and San Antonio.** The trap data for all south Texas locations and all San Antonio locations are averaged.

<https://doi.org/10.1371/journal.pntd.0014005.g008>

Another concern with a large by-catch is the potential ecological impact by removing insects that perform essential ecological services in an environment. Fortunately, we noticed very few honeybees (1,193) in the traps over three years, so this is one beneficial insect not expected to be significantly impacted by the trap. Because Kissing Bug Kill Traps will most likely be used in or near to ecologically disturbed areas of human habitation, it is possible that a substantial portion of the by-catch will be comprised of insects that preferentially inhabit or invade such disturbed areas, many of which are considered to be pests, providing an additional benefit to the use of the traps. For example, a large number of the by-catch were Coleoptera, made up with a large proportion of click beetles (Elateridae). Although we did not identify the click beetles to species, many elaterid larvae (wireworms) are major pests worldwide [30]. Even if the by-catch were comprised solely of insects that provided beneficial ecological services, we also contend that the impact of the traps would be inconsequential on a landscape level. Even if traps were used in large-scale surveillance or mass-trapping programs they would impact a minute area compared to the entire landscape. Moreover, the easily quantifiable removal of non-target insects from the environment caused by Kissing Bug Kill Traps would represent a tiny fraction of the apparently huge (but hard to quantify) impacts posed by large-scale habitat alterations, such as clearing forests for agriculture or urban development [31], or the non-target impact of annual worldwide application of 3 billion kg of pesticides [32]. Unlike chemical pesticides [33,34], the

Kissing Bug Kill Trap would leave no long-term environmental residue. Such anthropogenic activities as habitat degradation and non-target impact of pesticides are hypothesized to be one of the major drivers of the alarming global decline in insect populations [35,36].

If a future goal is to minimize trap by-catch, the collection cups of Kissing Bug Kill Traps could be modified to minimize by-catch. For example, Kissing Bug Kill Trap experiments in 2025 used dry collection cups, with no liquid preservatives. If a small hole was placed in these dry collection cups, arthropods smaller than adult triatomines (the vast majority of the by-catch) could escape unharmed. Additionally, frequent monitoring of traps with dry collection cups could allow non-target arthropods to be released alive while retaining only the triatomines.

Of the *T. gerstaeckeri* captured, 74% were males. This result is similar to that reported for *T. infestans* and *T. eratyrsiformis* in Argentina, where more flying males were captured using light traps than females, but not for *T. guasayana*, wherein 62% were female [2]. Fimbres-Macias, Harris [37] collected 1.6x more female than male *T. sanguisuga* manually around a building in Texas, USA, with light as an attractant. With 3,215 triatomines collected from a citizen science program, more females than males were collected for *T. gerstaeckeri*, *T. sanguisuga*, *T. indictiva*, *T. rubida*, while more males than females were collected for *P. lecticularia*, *T. protracta*, and *H. neotomae* [24]. Collectively, these results indicate that the sex ratio of triatomines attracted by light may differ among species and may differ from the sex ratio of natural populations.

Our previous study detected *T. cruzi* in triatomines captured by Kissing Bug Kill Traps [11], demonstrating that propylene glycol is suitable for preservation of nucleic acids. The current study represents our first attempt at using bloodmeal metabarcoding [38] to identify prior hosts of triatomines captured in Kissing Bug Kill Traps. One of the two captured *T. dimidiata* subjected to the bloodmeal analysis had evidence of past feeding on dog and chicken blood, suggesting that preservation in propylene glycol is suitable for this analysis. Similarly, with a larger set of triatomines collected alive manually from houses in the same neighborhoods, Juarez, Moller-Vasquez [39] found chicken and dog as frequent bloodmeal hosts, but also detected evidence of prior feeding on *Rattus rattus*, *Felis catus*, *Homo sapiens*, *Mus musculus*, *Sus scrofa*, Anatidae and *Archimandrita* sp. These bloodmeal results from the two insects caught in the Kissing Bug Kill Traps in our study suggest that they emerged from a domestic habitat, prior to flying and being intercepted by the trap, as opposed to a sylvatic habitat where wildlife hosts would be expected hosts. In the future, such assays could be used to identify the habitat of origin of dispersing adults, which would be important in understanding not only movement of triatomines but also the origins and prevalence of *T. cruzi*.

Our results build on the initial development of the Kissing Bug Kill Trap [11], and suggest that because of the efficacy, low cost, ease of maintenance, and durability of the trap it has promising potential for use as a large-scale surveillance tool. The trap would enable standardized passive surveillance methodology, avoiding observer bias that accompanies active sampling techniques. The multiple funnel design and solar-powered LED lights resulted in minimal trap failure over the three-year study, with only one instance of theft involving a solar-powered LED light. Most homeowners, especially in Guatemala which lacked electricity, appreciated the traps because they offered a free security light at night. The Kissing Bug Kill Trap may also have promise as a control tool to prevent dispersing triatomines from colonizing residences or domestic animal harborages thereby reducing the global threat of Chagas disease. The possibility of use as a control tool is supported by a 1954 study in which three light traps around an Arizona home captured 398 *Triatoma protracta* and the homeowners noticed fewer triatomines around their residence [8]. Deploying multiple traps around a household has proven to be effective in protecting humans from mosquito-borne pathogens [40]. The potential for practical implementation of the Kissing Bug Kill Trap awaits commercialization of this trap and its introduction into the pest control marketplace.

## Supporting information

### S1 Table. Arthropod bycatch for the different multi-funnel trap prototypes used in 2022–2024.

(XLSX)

**S2 Table. The number of triatomines and triatomines per day per trap (in parenthesis) of the six species captured from 2022-2024 in Texas and Guatemala.**

(XLSX)

**S1 Fig. Examples of processing by-catch arthropods from Kissing Bug Kill Traps after a week of collection.** Triatomines were removed and counted as the first step as they are larger than most of the by-catch and have a distinctive shape.

(TIFF)

**S2 Fig. Example of the collected arthropods, including two adult triatomines, from a Kissing Bug Kill Trap deployed for one week (A) and an example of the floating method to speed-up processing of large numbers of small arthropod by-catch (B).**

(TIFF)

**S1 Data. Dataset used for the analyses in this study.**

(XLSX)

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## Author contributions

**Conceptualization:** Sarah A. Hamer, John H. Borden, Michael G. Banfield, Gabriel L Hamer.

**Data curation:** Yuexun Tian, Nadia A. Fernández-Santos, Sarah C. Sittenauer, Claire C. Nevins.

**Formal analysis:** Yuexun Tian, Sujata Balasubramanian.

**Funding acquisition:** Pamela Pennington, Michael G. Banfield, Norma Padilla, Gabriel L Hamer.

**Investigation:** Yuexun Tian, Nadia A. Fernández-Santos, Henry Esquivel, Andrea M. Moller-Vasquez, María Granados-Presa, Adriana Echeverria, Alejandra Zamora-Jerez, Juan P. Fimbres-Macias, Walter Roachell, Paul A. Lenhart, Theresa Casey, Molly E. Keck, Carolyn L. Hodo, Christopher H. Downs, Sarah C. Sittenauer, Claire C. Nevins, Sujata Balasubramanian, Carlos Angulo, Carlos Palacios-Cardiel, Ramon Gaxiola-Robles, Tania Zenteno-Savín.

**Methodology:** Sarah A. Hamer, John H. Borden, Michael G. Banfield, Gabriel L Hamer.

**Visualization:** Yuexun Tian.

**Writing – original draft:** Yuexun Tian.

**Writing – review & editing:** Yuexun Tian, Nadia A. Fernández-Santos, Jose G. Juarez, Henry Esquivel, Andrea M. Moller-Vasquez, María Granados-Presa, Adriana Echeverria, Pamela Pennington, Alejandra Zamora-Jerez, Juan P. Fimbres-Macias, Walter Roachell, Paul A. Lenhart, Theresa Casey, Molly E. Keck, Carolyn L. Hodo, Christopher H. Downs, Sarah C. Sittenauer, Claire C. Nevins, Sujata Balasubramanian, Carlos Angulo, Carlos Palacios-Cardiel, Ramon Gaxiola-Robles, Tania Zenteno-Savín, Sarah A. Hamer, John H. Borden, Michael G. Banfield, Norma Padilla, Gabriel L Hamer.

## References

1. Reynoso MMN, Seccacini EA, Calcagno JA, Zerba EN, Alzogaray RA. Toxicity, repellency and flushing out in *Triatoma infestans* (Hemiptera: Reduviidae) exposed to the repellents DEET and IR3535. *PeerJ*. 2017;5:e3292. <https://doi.org/10.7717/peerj.3292> PMID: [28533956](https://pubmed.ncbi.nlm.nih.gov/28533956/)

2. Abrahan LB, Gorla DE, Catalá SS. Dispersal of *Triatoma infestans* and other Triatominae species in the arid Chaco of Argentina: flying, walking or passive carriage? The importance of walking females. *Mem Inst Oswaldo Cruz*. 2011;106(2):232–9. <https://doi.org/10.1590/s0074-02762011000200019> PMID: 21537686
3. Lorenzo MG, Reisenman CE, Lazzari CR. *Triatoma infestans* can be captured under natural climatic conditions using yeast-baited traps. *Acta Trop*. 1998;70(3):277–84. [https://doi.org/10.1016/s0001-706x\(98\)00042-4](https://doi.org/10.1016/s0001-706x(98)00042-4) PMID: 9777714
4. Noireau F, Abad-Franch F, Valente SAS, Dias-Lima A, Lopes CM, Cunha V, et al. Trapping Triatominae in silvatic habitats. *Mem Inst Oswaldo Cruz*. 2002;97(1):61–3. <https://doi.org/10.1590/s0074-02762002000100009> PMID: 11992149
5. Noireau F, Flores R, Vargas F. Trapping sylvatic Triatominae (Reduviidae) in hollow trees. *Trans R Soc Trop Med Hyg*. 1999;93(1):13–4. [https://doi.org/10.1016/s0035-9203\(99\)90161-x](https://doi.org/10.1016/s0035-9203(99)90161-x) PMID: 10492778
6. Wisnivesky-Colli C, Gürtler RE, Solarz ND, Schweigmann NJ, Pietrokovsky SM, Alberti A, et al. Dispersive flight and house invasion by *Triatoma guasayana* and *Triatoma sordida* in Argentina. *Mem Inst Oswaldo Cruz*. 1993;88(1):27–32. <https://doi.org/10.1590/s0074-02761993000100006> PMID: 8246755
7. Minoli SA, Lazzari CR. Take-off activity and orientation of triatomines (Heteroptera: Reduviidae) in relation to the presence of artificial lights. *Acta Trop*. 2006;97(3):324–30. <https://doi.org/10.1016/j.actatropica.2005.12.005> PMID: 16460653
8. Sjogren RD, Ryckman RE. Epizootiology of *Trypanosoma cruzi* in southwestern North America. 8. Nocturnal flights of *Triatoma protracta* (Uhler) as indicated by collections at black light traps (Hemiptera: Reduviidae: Triatominae). *J Med Entomol*. 1966;3(1):81–92. <https://doi.org/10.1093/jmedent/3.1.81> PMID: 5941571
9. Updyke EA, Allan BF. An Experimental Evaluation of Cross-Vane Panel Traps for the Collection of Sylvatic Triatomines (Hemiptera: Reduviidae). *J Med Entomol*. 2018;55(2):485–9. <https://doi.org/10.1093/jme/tjx224> PMID: 29272499
10. Rebollar-Téllez EA, Reyes-Villanueva F, Escobedo-Ortegón J, Balam-Briceño P, May-Concha I. Abundance and nightly activity behavior of a sylvan population of *Triatoma dimidiata* (Hemiptera: Reduviidae: Triatominae) from the Yucatan, México. *J Vector Ecol*. 2009;34(2):304–10. <https://doi.org/10.1111/j.1948-7134.2009.00038.x> PMID: 20836833
11. Hamer GL, Fimbres-Macias JP, Juárez JG, Downs CH, Carbajal E, Melo M, et al. Development of an operational trap for collection, killing, and preservation of triatomines (Hemiptera: Reduviidae): the kissing bug kill trap. *J Med Entomol*. 2024;61(6):1322–32. <https://doi.org/10.1093/jme/tjae087> PMID: 39024462
12. Lindgren BS. A multiple funnel trap for scolytid beetles (Coleoptera). *Can Entomol*. 1983;115(3):299–302. <https://doi.org/10.4039/ent115299-3>
13. Pacheco-Tucuch FS, Ramirez-Sierra MJ, Gourbière S, Dumontel E. Public street lights increase house infestation by the Chagas disease vector *Triatoma dimidiata*. *PLoS One*. 2012;7(4):e36207. <https://doi.org/10.1371/journal.pone.0036207> PMID: 22558384
14. Otálora-Luna F, Pérez-Rondón O, Iniciarte I, Aldana E. Ammonia and colors as attractants to *Rhodnius prolixus* (Hemiptera: Reduviidae, triatominae). *Saber*. 2018;30:253–64.
15. Reisenman CE, Lazzari C. Spectral sensitivity of the photonegative reaction of the blood-sucking bug *Triatoma infestans* (Heteroptera: Reduviidae). *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 2006;192(1):39–44. <https://doi.org/10.1007/s00359-005-0045-x> PMID: 16133499
16. Lent H, Wygodzinsky P. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. *Bulletin of the American Museum of Natural History*. 1979;163(3):123–520.
17. Bustamante DM, De Urioste-Stone SM, Juárez JG, Pennington PM. Ecological, social and biological risk factors for continued *Trypanosoma cruzi* transmission by *Triatoma dimidiata* in Guatemala. *PLoS One*. 2014;9(8):e104599. <https://doi.org/10.1371/journal.pone.0104599> PMID: 25170955
18. Juárez JG, Pennington PM, Bryan JP, Klein RE, Beard CB, Berganza E, et al. A decade of vector control activities: Progress and limitations of Chagas disease prevention in a region of Guatemala with persistent *Triatoma dimidiata* infestation. *PLoS Negl Trop Dis*. 2018;12(11):e0006896. <https://doi.org/10.1371/journal.pntd.0006896> PMID: 30399143
19. R Core Team. R language and environment for statistical computing. R Foundation for Statistical Computing. 2020.
20. Balasubramanian S, Hamer G, Hamer S. Bloodmeal metabarcoding of *Triatoma dimidiata* from Guatemala. *Dryad*. 2025. <https://doi.org/10.5061/dryad.pzgmsbczg>
21. de Paiva VF, de Oliveira J, Belintani T, Galvão C, Gil-Santana HR, da Rosa JA. *Hospesneotomae* n. gen. of the Triatomini tribe presents a turn-around in the taxonomy of the *Triatoma protracta* species complex. *Sci Rep*. 2025;15(1):8143. <https://doi.org/10.1038/s41598-025-91399-w> PMID: 40059180
22. Miller DR, Crowe CM, Barnes BF, Gandhi KJK, Duerr DA. Attaching lures to multiple-funnel traps targeting saproxylic beetles (Coleoptera) in pine stands: inside or outside funnels? *J Econ Entomol*. 2013;106(1):206–14. <https://doi.org/10.1603/ec12254> PMID: 23448033
23. Curtis-Robles R, Wozniak EJ, Auckland LD, Hamer GL, Hamer SA. Combining Public Health Education and Disease Ecology Research: Using Citizen Science to Assess Chagas Disease Entomological Risk in Texas. *PLoS Negl Trop Dis*. 2015;9(12):e0004235. <https://doi.org/10.1371/journal.pntd.0004235> PMID: 26658425
24. Curtis-Robles R, Hamer SA, Lane S, Levy MZ, Hamer GL. Bionomics and Spatial Distribution of Triatomine Vectors of *Trypanosoma cruzi* in Texas and Other Southern States, USA. *Am J Trop Med Hyg*. 2018;98(1):113–21. <https://doi.org/10.4269/ajtmh.17-0526> PMID: 29141765
25. Mérida Ruíz SA, Guerra Centeno DS, Bailey Leonardo EL, Rohn K, Kösters S, Kreienbrock L. Participatory epidemiology at the neotropics: study of diseases of backyard livestock and description of hunting patterns in Uuxactún, Maya Reserve Biosphere, Guatemala. *BMC Res Notes*. 2016;9:207. <https://doi.org/10.1186/s13104-016-2009-3> PMID: 27055652

26. Frost SW. Insects Taken in Light Traps at the Archbold Biological Station, Highlands County, Florida. *The Florida Entomologist*. 1964;47(2):129. <https://doi.org/10.2307/3493289>
27. Johnson CG. Migration and dispersal of insects by flight. 1969.
28. Cohnstaedt LW, Lado P, Ewing R, Cherico J, Brabec D, Shults P, et al. Conceptualization, design, and construction of a novel insect mass trapping device: the USDA Biomass Harvest Trap (USDA-BHT). *J Econ Entomol*. 2024;117(4):1269–72. <https://doi.org/10.1093/jee/toae129> PMID: [38963914](https://pubmed.ncbi.nlm.nih.gov/38963914/)
29. Hribar LJ. Expanding basic entomological knowledge by using mosquito surveillance bycatch. *Ann Entomol Soc Am*. 2020;113(6):439–46.
30. Traugott M, Benefer CM, Blackshaw RP, van Herk WG, Vernon RS. Biology, ecology, and control of elaterid beetles in agricultural land. *Annu Rev Entomol*. 2015;60:313–34. <https://doi.org/10.1146/annurev-ento-010814-021035> PMID: [25341096](https://pubmed.ncbi.nlm.nih.gov/25341096/)
31. Wagner DL. Insect Declines in the Anthropocene. *Annu Rev Entomol*. 2020;65:457–80. <https://doi.org/10.1146/annurev-ento-011019-025151> PMID: [31610138](https://pubmed.ncbi.nlm.nih.gov/31610138/)
32. Carvalho FP. Pesticides, environment, and food safety. *Food and Energy Security*. 2017;6(2):48–60. <https://doi.org/10.1002/fes3.108>
33. Serrão JE, Plata-Rueda A, Martínez LC, Zancuncio JC. Side-effects of pesticides on non-target insects in agriculture: a mini-review. *Naturwissenschaften*. 2022;109(2):17. <https://doi.org/10.1007/s00114-022-01788-8> PMID: [35138481](https://pubmed.ncbi.nlm.nih.gov/35138481/)
34. Bartling M-T, Brandt A, Hollert H, Vilcinskis A. Current Insights into Sublethal Effects of Pesticides on Insects. *Int J Mol Sci*. 2024;25(11):6007. <https://doi.org/10.3390/ijms25116007> PMID: [38892195](https://pubmed.ncbi.nlm.nih.gov/38892195/)
35. Forister ML, Pelton EM, Black SH. Declines in insect abundance and diversity: We know enough to act now. *Conserv Sci and Prac*. 2019;1(8). <https://doi.org/10.1111/csp2.80>
36. Goulson D. The insect apocalypse, and why it matters. *Curr Biol*. 2019;29(19):R967–71. <https://doi.org/10.1016/j.cub.2019.06.069> PMID: [31593678](https://pubmed.ncbi.nlm.nih.gov/31593678/)
37. Fimbres-Macias JP, Harris TA, Hamer SA, Hamer GL. Phenology and environmental predictors of *Triatoma sanguisuga* dispersal in east-central Texas, United States. *Acta Trop*. 2023;240:106862. <https://doi.org/10.1016/j.actatropica.2023.106862> PMID: [36787862](https://pubmed.ncbi.nlm.nih.gov/36787862/)
38. Balasubramanian S, Curtis-Robles R, Chirra B, Auckland LD, Mai A, Bocanegra-Garcia V, et al. Characterization of triatomine bloodmeal sources using direct Sanger sequencing and amplicon deep sequencing methods. *Sci Rep*. 2022;12(1):10234. <https://doi.org/10.1038/s41598-022-14208-8> PMID: [35715521](https://pubmed.ncbi.nlm.nih.gov/35715521/)
39. Juarez JG, Moller-Vasquez AM, Granados-Presa M, Pennington P, Padilla N, Balasubramanian S, et al. *Triatoma dimidiata*, domestic animals and acute Chagas disease: a 10-year follow-up after an eco-bio-social intervention. *Parasit Vectors*. 2025;18(1):253. <https://doi.org/10.1186/s13071-025-06897-7> PMID: [40616155](https://pubmed.ncbi.nlm.nih.gov/40616155/)
40. Barrera R, Harris A, Hemme RR, Felix G, Nazario N, Muñoz-Jordan JL, et al. Citywide Control of *Aedes aegypti* (Diptera: Culicidae) during the 2016 Zika Epidemic by Integrating Community Awareness, Education, Source Reduction, Larvicides, and Mass Mosquito Trapping. *J Med Entomol*. 2019;56(4):1033–46. <https://doi.org/10.1093/jme/tjz009> PMID: [30753539](https://pubmed.ncbi.nlm.nih.gov/30753539/)