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Research Article

A Pilot Radio Telemetry Field Study of Triatomine Vectors (Hemiptera: Reduviidae) of the Chagas Disease Parasite

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Abstract

We conducted the first pilot radio telemetry study of hematophagous arthropods by placing transmitters on wild-caught triatomine insects ('kissing bugs'), vectors of the Chagas disease parasite. In Texas—a recognized hotspot for triatomine diversity and locally-acquired human and animal Chagas disease—we tagged five female and four male *Triatoma gerstaeckeri* (Stål) (Hemiptera: Reduviidae), as well as one female and one male *Triatoma sanguisuga* (Leconte) (Hemiptera: Reduviidae) in three counties from 2015 to 2017. In comparative trials, placement of the transmitter on the dorsal side of the abdomen underneath the hemelytra wings, with the transmitter antenna shortened to 3 cm, yielded the best results. We tracked the movements of the 11 tagged bugs over an average of 4.8 d (range of 1 to 12 d) and detected 18 movement events with an average distance of 3.8 m (range of 1 to 20 m). This pilot study demonstrates the potential utility for using telemetry as a tool for studying fine-scale non-flight movement of triatomines and the discovery of cryptic resting habitats. Future studies using this or similar technologies to study movement and behavior of triatomines could test for site-fidelity of resting habitats and provide novel insight into aspects of vector biology that could be targeted in disease risk reduction efforts.

Key words: behavior, Chagas disease, detection, monitoring, vector ecology

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*, which is estimated to infect over 5.7 million people throughout the Americas (World Health Organization 2015). The parasite is vectored by triatomine insects (Reduviidae: Triatominae), which feed on a wide variety of vertebrate hosts, including humans. Triatomines, known colloquially as 'kissing bugs' or 'conenose bugs', exist in sylvatic, peridomestic, and domestic environments throughout Central and South America. In the United States, triatomines rarely colonize human housing and instead human risk results when people enter sylvatic environments (e.g., through hunting, camping) or when adult triatomines disperse and are host-seeking in or near human homes (a problem amplified by suburban sprawl into previously sylvatic environments). Accordingly, studying the behavior of the dispersing adults in the United States is important to characterize when and where risk is highest and to help inform intervention campaigns aimed at reducing triatomine-human contact.

A limited understanding of triatomine dispersal was made possible by mark-release-recapture studies (MRR) (Ekkens 1981, Lehane and Schofield 1981, Schofield et al. 1992), morphometric studies (Dumonteil et al. 2007), and population genetic studies (Almeida et al. 2016). Compared to other hematophagous arthropods, however, triatomine dispersal is poorly understood; this is partly due to the unique features of their life history, which makes traditional MRR studies challenging. One feature is that triatomine density is typically much lower than that of other hematophagous arthropods, especially species of Culicidae. Mosquito population sizes based on MRR experiments vary greatly based on species and study area but typically produce estimates between 100,000 and 600,000 individuals per km^2 (Reisen et al. 1991, Costantini et al. 1996, Silver 2008). A recent review of mosquito MRR studies showed an average percent recapture of only 4%, when the average number of marked individuals released in a single event was 1,651 (Guerra et al. 2014). In contrast, the estimate of sylvatic triatomine density ranged from 24,700 to 38,400 individuals per km^2 based on estimates in southern Texas (Burkholder et al. 1980). A dispersal study of three different *Triatoma* spp. in Arizona released 821 marked bugs over the course of 2 yr and recaptured 44 (5.4% recapture rate) (Ekkens 1981). Given the expected low recapture success, a large number of marked triatomines would need to be released into a single location to study dispersal, which is not representative of a natural event, given triatomine biology. Another challenge with triatomine tracking is that adult dispersal behavior appears to have seasonal structure

(Curtis-Robles et al. 2018) and is temperature-dependent (Ekkens 1981). Accordingly, studying the movement of wild triatomines would ideally use a technology able to track individual bugs not contingent on a massive release.

Advancements in technology of radio telemetry have allowed many different arthropods to be tracked in recent years (Kissling et al. 2014). Currently, the smallest available transmitter is approximately 0.2 g, which has been used to track *Bombus* spp. (Latreille) (Hymenoptera: Apidae) (Hagen et al. 2011), *Corydalus cornutus* (Linnaeus) (Megaloptera: Corydalidae) (Hayashi and Nakane 1989), and *Cordulegaster erronea* (Hagen) (Odonata: Cordulegastridae) (Moskowitz and May 2017). To date, however, radio telemetry has not been used to study the movements of arthropod vectors. We conducted pilot studies from 2015 to 2017 by tagging and tracking two species of wild triatomines in Texas. Here, we present the findings and evaluate the potential for radio telemetry to be used for the study of the biology of these vectors to improve our ability to minimize bridge transmission of the Chagas disease parasite to humans or domestic animals.

Materials and Methods

We conducted pilot telemetry field trials in Texas over the course of 3 yr using two species of wild-caught triatomines. Three localities—one each in Uvalde, Brazos, and Hidalgo, Counties—were utilized for this study.

In July 2015, we placed transmitters on four *Triatoma gerstaeckeri* (Stål) (Hemiptera: Reduviidae) found outside of a private residence in Uvalde County in west Texas. The location in Uvalde County was situated within the Edwards Plateau ecoregion, which is characterized by juniper-oak savanna and mesquite-oak savanna (Griffith et al. 2004). The site of triatomine capture and tracking was in the immediate vicinity of a home with ample outdoor night lighting set in a cleared area surrounded by dense thickets of shrubs and cactus. Two outdoor dogs slept on the side porch area of the home. Neighboring properties had livestock and irrigated agricultural usage.

In September 2016, we placed transmitters on two *Triatoma sanguisuga* (Leconte) (Hemiptera: Reduviidae) and one *T. gerstaeckeri* outside of a private residence in Brazos County in central Texas. The location in Brazos County was situated within the East Central Texas Plains ecoregion, which was historically characterized by post oak savanna vegetation. The site of triatomine capture and tracking was in the immediate vicinity of a rural home surrounded by an oak forest (*Quercus* spp.) with yaupon (*Ilex vomitoria*) as the dominant understory shrub.

In May 2017, we placed transmitters on four *T. gerstaeckeri* outside of a private residence in Hidalgo County in south Texas along the United States–Mexico border. The location in Hidalgo County was situated within the Western Gulf Coastal Plain ecoregion, which is characterized by mainly grasslands, with forest vegetation further inland. The site of triatomine capture and tracking was in the immediate vicinity of a home with ample outdoor night lighting set in a small clearing surrounded by a forest with dense undergrowth of mesquite (*Prosopis* spp.) and cactus. A small dog kennel was located in the rear of the house, approximately 20 m from the back porch; the kennel was constructed of chain link fence and crumbling cement flooring, with a plastic 'igloo' type dog house. Dogs were kept in the kennel during the day but were taken indoors at night. Neighboring agricultural properties include sugarcane (*Saccharum* spp.). The owner of the Hidalgo County property had experienced several sudden unexplained deaths of young dogs at the property; one of the dogs was diagnosed post-mortem with *Trypanosoma cruzi* infection. Subsequent to learning about Chagas disease and triatomines, the property owners reduced disease transmission using a multi-prong approach: minimizing outdoor exposure of their dogs, removing harborage sites around the outdoor kennel area, treating the area with insecticides, and active collection of triatomines. From 2013 to 2017, the owners collected >500 triatomines from the area around their home.

Locations were selected based on prior experience collecting at these locations to confirm bugs could be captured when equipment and personnel were available for the study. Wild triatomines were captured via a combination of black lights and manual searches of peridomestic environments (Curtis-Robles et al. 2018). Transmitters were applied upon capture, and triatomines were released back to the location of capture within 10 min. Transmitter application could be accomplished by one person, but having a second person to lift the wings with forceps helped facilitate placement of the transmitter on the abdomen.

We used the smallest glue-on transmitter available, which was a 0.2 g transmitter with a 10 cm silver thread antenna (model A2412; Advanced Telemetry Systems, Inc. Isanti, MN). This model of transmitter had an approximate battery life of 16 d, with a warranty life of 8 d. In 2015, we mounted transmitters with superglue to the dorsal side of the thorax (pronotum) on two individuals and also to the dorsal side of the abdomen on two other individuals (Fig. 1). For two tagged bugs we used the full 10-cm transmitter antenna and for two others we cut the antenna to a length of 3 cm—similar to other studies (Rink and Sinsch 2007, Chiari et al. 2013, Tini et al. 2018)—which did not affect wavelength. The attachment location on the abdomen appeared to interfere less with the wings and was also more streamlined, and the shorter antenna length of 3 cm appeared to be less intrusive to bug movement. Accordingly, we continued with this approach of shorter antenna length glued to the dorsal side of the abdomen in 2016 and 2017. Prior to attachment, transmitters were painted with blue, red, or yellow luminous paint (Catalog 1166A BioQuip Products, Rancho Dominguez, CA). A UV blacklight flashlight (WF-502B, 395-410nm, Alldaymall.com) was used at night to see the painted transmitters mounted to kissing bugs. In 2015 and 2016, we used Loctite Super Glue, ULTRA Gel Control (Henkel Corporation, Rocky Hill, CT) and in 2017, we used Gorilla Super Glue Gel (The Gorilla Glue Company, Cincinnati, OH).

The tagged bugs were tracked using a receiver (Model R410) and a three-element folding Yagi Antenna (Advanced Telemetry Systems, Inc. Isanti, MN; Fig. 2). Tracking techniques included the 'homing' as a method to use antenna directionality and signal strength to move toward the transmitter with the goal of making visual contact (reviewed by Samuel and Fuller 1996). We obtained a telemetry location using the homing-in method on multiple days post-release until one of the following events occurred: 1) the triatomine died, 2) the transmitter battery died, or 3) the transmitter signal was lost and transmitter was unable to be recovered. When we lost a signal, attempts to recover the signal were carried out in different directions out to about 0.5 km from the last known location. Tracking efforts were focused both during the day—to identify resting habitats—and around dusk and several hours following dusk, which is when triatomines typically become active (Sjogren and Ryckman 1966, Ekkens 1981).

We did not have a sensitive scale in the field to weigh triatomines prior to placement of transmitters. Instead, we estimated the ratios of transmitter weight to triatomine body weight, using adult triatomines from our nascent triatomine colony, which were either wild-caught or progeny of wild-caught specimens from Texas. We

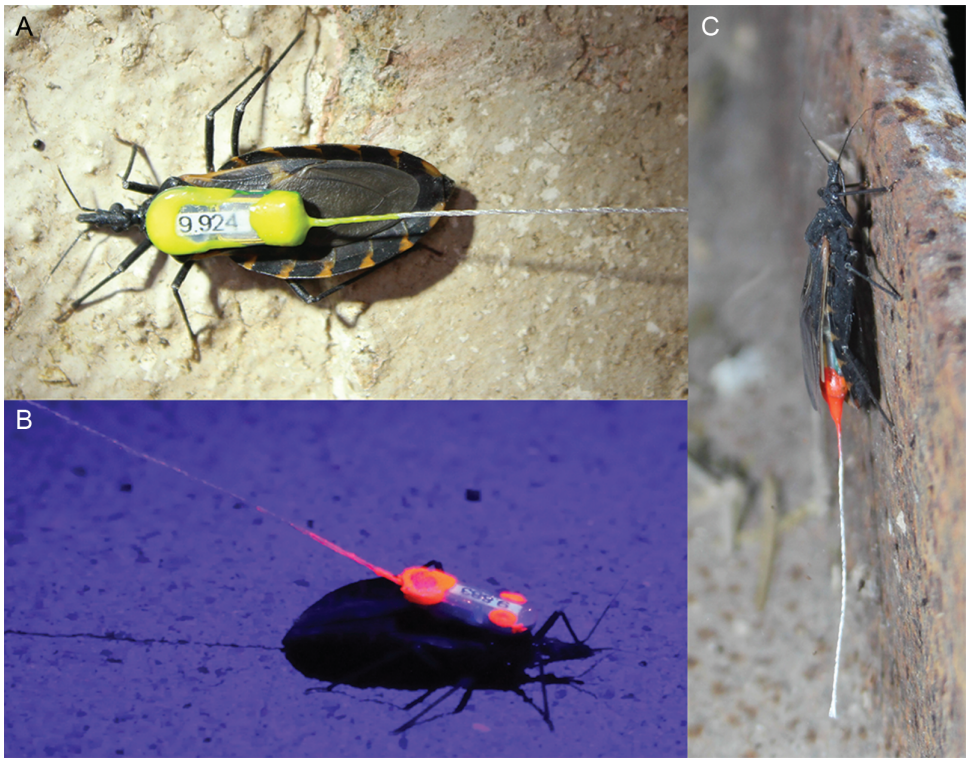

Fig. 1. *Triatoma gerstaeckeri* with radio transmitters. (A) Transmitter mounted to the dorsal side of the thorax, (B) the appearance of the fluorescent paint under ultraviolet light, and (C) the transmitter mounted to the dorsal side of the abdomen with a shortened, 3 cm antenna. The radio-tagged triatomines in panel A and C were on vertical surfaces.

Fig. 2. Tracking tagged *Triatoma gerstaeckeri* in a dog kennel in Hidalgo County Texas. (A) The circle inset is the connection of the two shelves of this plastic dog house which is where the cryptic habitat was utilized and (B) a tagged female discovered in a cryptic hiding location on a vertical surface inside the dog kennel.

weighed four *T. gerstaeckeri* females, three *T. gerstaeckeri* males, and two *T. sanguisuga* females. These specimens had all been provided an artificial bloodmeal in the prior week but were not obviously blood engorged at the time of weighing. We calculated mean weights and standard errors.

Results

The first trial, in Uvalde County, started 13 July 2015 and resulted in radio-tagging four adult *T. gerstaeckeri* collected from the front porch of a private residence between 2230 and 0110 hours; two were female and two were male. The next day, one of the males was discovered dead in the grass about 3 m from the site of release (Table 1). It was apparent that the original 10-cm antenna on this transmitter had become tangled in the grass. One female moved 2 m during the first night to a crack in the base of the house foundation out of sight and remained in that location for the next 4 d with a signal after which the signal was no longer detected. The remaining male and female were re-discovered on the porch on the morning following the application of the transmitter; they had moved 2 m and 3 m, respectively. At around 2300 hours these two bugs remained alive and in the same locations on the front porch concrete; by the third day, they were in the same locations, but appeared lethargic and eventually were found dead on the fourth day (Table 1). After observing these triatomines die and subsequently seeing numerous arthropods, including triatomines, dead around the base of the home, conversations with the home owners revealed that several months prior the home had been treated with a residual insecticide by a pest management professional.

The second trial, in Brazos County, started 11 September 2016, during which we radio-tagged one male and one female *T. sanguisuga* near the porch of a private residence between 2230 and 2320 hours. The following night we captured, tagged, and released one male *T. gerstaeckeri*. All three triatomines were captured and released near the outer wall of the house where the plastic siding on the second story intersected with the first story brick siding, creating a flat surface for the bug to reorient itself. This location was approximately 2.5 m above the ground and had access to a crevice underneath the house siding that allowed enough space for kissing bugs to crawl between. The female *T. sanguisuga* released at this interface near the siding immediately crawled under the siding, moved an estimated 1 m horizontally on the side of the house the second night, and then remained in that location for the following 4 d (Table 1). The male *T. sanguisuga* moved an estimated 1 m behind the siding the next night and an additional 1 m behind the siding by the fifth night. We did not check the signal after the fifth day. The male *T. gerstaeckeri* was discovered the following night dead and entangled in thick foliage, with suspected chew marks on the posterior process of the scutellum and hemelytra wings.

The third trial, in Hidalgo County, started 8 May 2017, during which we radio-tagged one male and three female *T. gerstaeckeri* around the rural property of a private residence between 2100 and 2200 hours. The male individual was found, tagged, and released near a metal shed. After one day, the male bug moved 1 m to the inside of the wall of a metal shed and stayed in that location for 3 more days (Table 1). Tracking was not possible on day 5 or 6, and during the next attempt on day 7, the signal was not found after extensive searching, suggesting a dead battery or possibly the dispersal out of range. The first female was captured between the wooden deck and the brick home and released at that location, moved 4 m along the side of the house immediately after release, and then 24 h later, was back at the site of release under the corner of the back porch. Three days after release, this female had moved 10 m to the opposite side of the porch but remained hidden from sight, where the signal remained in that location on day 7 and day 9, but there was no detectable signal on day 12. The second female was captured and released on the back porch area. During the first night, she moved 4 m under the back porch deck and remained in that location when checked on days 3, 7, 9, and 12 until there was no detectable signal on day 14. The third female was captured and released on the dog kennel floor, where she was found 0.5 m away in the joint between the top and bottom portion of the dog house 24 h later (Fig. 2; Supp. Video S1). By day 3, she had moved 1 m outside the kennel in a crevice next to a pipe. By day 7, she had moved about 20 m from the dog kennel to the porch next to the house. On day 9, the signal from this third female was lost.

Table 1. Details on the 11 radio-tagged triatomines in Texas from 2015 to 2017

Bug ID	County, year	Species, sex	Start date	End date	Summary
1	Uvalde, 2015	*T. gerstaeckeri*, F	13 July 2015	17 July 2015	Moved 2 m first night, remained in location 4 d, not recovered (owner disclosed insecticide use).
2	Uvalde, 2015	*T. gerstaeckeri*, F	13 July 2015	16 July 2015	Moved 3 m first night, remained in location 3 d before dying (owner disclosed insecticide use).
3	Uvalde, 2015	*T. gerstaeckeri*, M	13 July 2015	14 July 2015	Moved 3 m first night, antenna tangled in grass and found dead (owner disclosed insecticide use).
4	Uvalde, 2015	*T. gerstaeckeri*, M	13 July 2015	16 July 2015	Moved 2 m first night, then 5 m second day and found dead (owner disclosed insecticide use).
5	Brazos, 2016	*T. sanguisuga*, F	11 Sept. 2016	16 Sept. 2016	Remained in location first night, moved 1 m second night, remained in same location on day 5 and never re-checked.
6	Brazos, 2016	*T. sanguisuga*, M	11 Sept. 2016	16 Sept. 2016	Move 1 m first night, moved 1 m by day 5 and never re-checked.
7	Brazos, 2016	*T. gerstaeckeri*, M	12 Sept. 2016	13 Sept. 2016	Found in grass after day 1 with bite marks.
8	Hidalgo, 2017	*T. gerstaeckeri*, F	8 May 2017	19 May 2017	Moved 4 m at release, returned to release point next day, moved 10 m day 3, remained in location, signal lost on day 12.
9	Hidalgo, 2017	*T. gerstaeckeri*, F	8 May 2017	19 May 2017	Moved 4 m at release, stayed in location for 12 d and signal lost on day 14.
10	Hidalgo, 2017	*T. gerstaeckeri*, F	8 May 2017	16 May 2017	Moved ½ m into cryptic habitat inside plastic of dog kennel first night, moved 1 m day 3, moved 20 m to porch day 7, signal lost on day 9.
11	Hidalgo, 2017	*T. gerstaeckeri*, M	8 May 2017	12 May 2017	Moved 1 m first night, stayed in location for the next 3 d, signal lost on day 7.

In order to determine the approximate ratio of transmitter weight to insect weight, we measured wild caught or progeny of wild caught *T. gerstaeckeri* and *T. sanguisuga* individuals from our triatomine colony. The average weight of a *T. gerstaeckeri* adult female (n = 4) was 279.0 ± 54.9 mg (mean ± SE) and of an adult male (n = 3) was 212.6 ± 5.9 mg. The weight of the transmitter was 200 mg, which was 71.7% of the body weight of a female and 94.1% of the body weight of a male. The average weight of an adult female (n = 2) *T. sanguisuga* was 178.1 ± 0.02 mg, which means the transmitter was about 112.3% of the body weight.

Discussion

We conducted a pilot field study to explore the potential utility of studying the biology of wild triatomines using radio telemetry. Through these three field trials, we explored different transmitter mounting techniques and antenna lengths to attempt to minimize influence on triatomine behavior.

The effect of transmitter size/weight on triatomine movement was a concern. We focused the majority of our efforts on the largest species of *Triatoma* found in Texas, *T. gerstaeckeri* (Lent and Wygodzinsky 1979). In comparison to the 200 mg weight of the transmitter, *T. gerstaeckeri* adults have been documented as taking an average bloodmeal of 132.59 mg (male) to 217.68 mg (female) (Pippin 1970). We therefore expected that an additional 200 mg on a triatomine would not unduly hinder movement. Similarly, the transmitters for several studies tagging *Bombus* spp., *Lucanus cervus*, *C. cornutus* ranged from 35 and 185% of the weight of the insect (Hayashi and Nakane 1989, Rink and Sinsch 2007, Hagen et al. 2011, Kissling et al. 2014). However, given the potential of these transmitters to impact normal behavior, especially flight, a study evaluating the impact of the mounted transmitter on bug behavior is needed. Despite concerns for restricted movement, we did observe radio-tagged triatomines with normal ground locomotion including crawling on vertical surfaces (Supp. Video S1). This observation is interesting to consider in relation to potential bloodmeal weight—a recently blood fed triatomine would be expected to move fairly unhindered by the additional weight of a bloodmeal. We never observed radio-tagged triatomines attempting to fly; this was expected, given that transmitters were frequently attached in a way that restricted wing movement. However, based on our prior fieldwork, we have rarely observed triatomines in flight (e.g., adult triatomines attracted to CO_2 bait and black lights most commonly were observed to crawl to trapping stations, rather than fly) and we were, therefore, most interested in locomotion by walking.

We tracked the movements of the 11 tagged bugs over an average of 4.8 d with a range of 1 to 12 d. When the signal from these tagged bugs ceased and we were unable to recover the signal during a search of the surrounding area, the bug could have made a larger dispersal event or the transmitter battery died. The transmitters used in this study were manufactured with an approximate battery life of 16 d, with a warranty life of 8 d. We know at least one transmitter remained active for 12 d, but by the 14th day, the signal was no longer found. When these transmitters were exposed and in direct visual contact with the Yagi antenna, we were able to pick-up a signal approximately 100 m away. However, when tagged bugs were in their daytime resting locations under porches or in cracks in the ground, the antenna/receiver needed to be closer than 25 m to receive the signal. We did not attempt to elevate the antenna (e.g., on a tower or kite), but we suspect that would have increased the distance at which signal was detectable (Deppe et al. 2015). Future studies comparing the transmitter antenna length and effective radiated power are warranted.

We detected 18 movement events, with an average movement distance of 3.8 m (range of 1 to 20 m). Some of these movement events were likely representative of the fine-scale movement of triatomines and relative inactivity during their daytime resting location and nighttime host-seeking period (Guerenstein and Lazzari 2009, Lazzari and Lorenzo 2009). The use of luminous paints on the transmitters improved our ability to re-sight the triatomines during tracking; they allowed us to pick-up the visual at night while homing at a greater distance and also several visuals through cracks in the deck were only possible given the bright fluorescence with the use of the black light. During the 2017 trial, we tracked several bugs from just before sunset to a few hours after sunset. While periodically checking on their locations, we noticed them moving and obtained visuals of them crawling under or near the porch or in and around the dog kennel (Supp. Video S1). In addition, when the tagged bugs were initially released, some moved several meters away from the point of capture. Then the day following release, the bugs had returned to hiding places near the point of capture. This leads us to believe that these triatomines exhibited site fidelity for these daytime resting refugia, as has been supported by studies of the importance of fecal and cuticle chemical compounds to refugia homing in triatomines (Lorenzo and Lazzari 1996, Lorenzo Figueiras and Lazzari 1998). Other studies have also illustrated that triatomines are more likely to be attracted to dark spaces, and that olfactory cues such as feces may play a major role in site fidelity of triatomine bugs (Figueiras et al. 1994, Reisenman et al. 2000). The use of trail pheromones to relocate resting habitat is a possible mechanism to allow triatomines to return to safe resting sites. Future studies are needed to see if the movements observed in our pilot efforts deviate from random movement.

This study also led to the discovery of one resting habitat that was cryptic with respect to routine searching for triatomines. In the 2017 trial, while attempting to locate the female *T. gerstaeckeri* inside the dog kennel on the second night after release, the signal was coming from the kennel but the insect was unable to be seen. After searching under the doghouse, inside it, and under the pad and water bowl, the bug remained hidden. Only when we moved the doghouse did we realize the signal remained strongest with the relocated doghouse. We separated the doghouse into the top and bottom halves and discovered the tagged bug was alive and concealed inside the small space where the two halves of the doghouse join together (Fig. 2). Given the extensive history of canine Chagas disease at this property (see Methods section), the owner of these dogs routinely looks for triatomines inside the kennel as well as in and under the doghouse, but without dismantling the doghouse. Therefore, this triatomine was in a cryptic location that would have been missed during routine inspection. The discovery of this cryptic resting habitat is similar to a recent study, which used tagged 'judas beetles' to find cryptic breeding sites of coconut rhinoceros beetles (Scarabaeidae) (Moore et al. 2017).

One unexpected observation during the first trial in 2015 in Uvalde County was the mortality of the tagged bugs, likely due to the residual insecticide used to treat the home. In this respect, releasing tagged bugs could be used as an evaluation to see if different active ingredients successfully kill triatomines in the field. This would be a particularly important trial given that no compounds are labeled for use to control triatomines in the United States. This approach is similar to the way in which colony mosquitoes are caged during field trials to evaluate knockdown of ultra-low volume treatment (Dennett et al. 2017). The deployment of tagged triatomines could

be a tool for evaluating intervention campaigns using insecticides such as paint containing microencapsulated insecticide (Gorla et al. 2015) and other long-lasting insecticides.

Advances in telemetry technology might allow transmitter and battery sizes to continue to shrink, allowing a less invasive tool to track the movement of triatomines. This pilot study helps to evaluate the utility of telemetry as a tool for studying fine-scale movement of triatomines, begin to understand patterns of site use, and discover cryptic resting habitats.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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