Scientific Note

An effective sampling tool for adult crabhole inhabiting *Deinocerites* mosquitoes

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The mosquito genus *Deinocerites* is a monophyletic assemblage encompassing 18 Neotropical crabhole habitat-specialist taxa that cluster into five phylogenetically interrelated, but morphologically distinct, groupings (Dyar and Knab 1915, Lane 1953, Adames 1971). Immature stages of *Deinocerites* develop in water that accumulates inside the holes excavated by crabs, including those in the genera *Epilobocera*, *Uca*, *Cardisoma,* and *Gecarcinus*. Adult *Deinocerites* rest inside the hole during the day, become active at night and move outside, but only for short appetential or mating flights (Downes 1966). Females of *Deinocerites* regularly bite humans, but they are opportunistic feeders that take blood from a great variety of vertebrates, including reptiles, amphibians, birds, and both domestic and wild mammals (Tempelis and Galindo 1970, Edman 1974).

Overall, these mosquitoes are not considered to be a public health threat, but some species are occasionally infected with arboviruses, including Venezuelan equine encephalitis and St. Louis encephalitis (Tempelis and Galindo 1970, Adames 1971). There have been a few studies of *Deinocerites* ecology and behavior that identified species using larval, pupal, and adult morphological characters as well as recognizing initial phylogenetic relationships and biogeographic history (Adames 1971, Carpenter and LaCasse 1955, Adames and Hogue 1969). However, the lack of studies about this bizarre and yet biologically fascinating group of mosquitoes is without question due to their poor role as carriers of human pathogens (Horsfall 1955). Nonetheless, this could also be the result of ineffective tools for sampling different life stages (Lane 1953).

Among the subjects requiring investigation in crabhole inhabiting *Deinocerites* are the species coexistence and abundance patterns. These topics could be tackled by sampling immature stages of *Deinocerites* (e.g., larvae and pupae) directly from the water of crabholes, but collecting and rearing these stages to adulthood is normally complicated. Furthermore, morphological keys to identify larvae and pupae are not as discriminatory as the ones used to identify adult specimens (Adames 1971). One way to overcome these difficulties is by sampling adult *Deinocerites* with manual aspirators when they are resting inside crabholes (Heinemann and Belkin 1978). However, this task could be unrealistic in some cases, as it depends on hole dimension and depth, which might vary greatly among different sites (O'Meara and Mook 1990). Herein, we describe a simple and effective apparatus to collect adult resting *Deinocerites* from the interior of crab holes.

To test our sampling device, we visited six localities across Panama, four on the Pacific and two on the Atlantic coasts (Table 1). Adult mosquitoes were collected consecutively for seven days in each locality and morphologically identified using the taxonomic key of Adames (1971). They were killed with chloroform, stored individually in 1.5 ml tubes, and placed inside plastic bags along with silica gel. Our domestic sampling apparatus consisted of three main parts: a 45-degree Y-shaped PVC fitting pipe connection, a floor-pump, and a collecting cage (Figure 1). The terminal section of the pump, including the air-blowing cylinder (i.e., A 40 cm long and 1 cm in diameter hosepipe), was introduced into one of the top openings of the Y-shaped PVC connection and attached to it through a 1.2 cm PVC threaded reducing bushing and a 1.9 cm Sch 40 PVC male adapter. The PVC male adapter connecting with the Y shape PVC connection was wrapped up at both ends with circular sections of fiberglass mesh to prevent mosquitoes from escaping through the air-blowing unit (Figure 1). A 1.2 m long wooden stick provided physical support to the pump and was attached with duct tape. The mosquito-collecting cage was secured to the other top opening of the Y-shaped PVC connection with a rubber band, and the bottom opening was placed directly on the ground to cover the entry of the crabhole. We introduced the air-blowing cylinder in each crabhole, pumped air inside roughly 25 times, and repeated this air-boosting procedure three times in each hole before moving into a different one. The hole entrance was covered in between air-pumping cycles to prevent mosquitoes from escaping. Mosquitoes were then extracted from the collecting cage using a manual aspirator and transferred into smaller individual cages.

We collected 1,623 *Deinocerites* mosquitoes in 309 arbitrarily surveyed crabholes from six different coastal areas of Panama, averaging 5.3 mosquitoes per hole. Only 12% of the holes were negative for the presence of mosquitoes. Six species from five of the biogeographic groupings proposed by Adames (1971) were collected with our device (Table 1). *Deinocerites cancer*, *De. epitedeus*, and *De. atlanticus* were gathered from localities on the Atlantic coast, whereas *De. spanius*, *De. pseudes,* and *De. diary* were collected from localities on the Pacific coast. The former two species were found co-occurring in the same crabholes in Bahia Azul, Bocas del Toro, while the rest were collected singly in their respective localities. *Deinocerites cancer* and *De. epitedeus* co-occurred in roughly 30% of the holes surveyed in Bahia Azul, and they also shared 50% of the niche with another unidentified mosquito species in the genus *Culex*. Regardless of the site, sex ratios were very similar across *Deinocerites* species, but body-size varied considerably within species, particularly in *De. pseudes*. Furthermore, many of the mosquitoes collected in this study were engorged with blood due to recent feeding.

Table 1. Species composition of *Deinocerites* mosquitoes collected from six localities across Panama using a domestic apparatus. Bold font indicates species that were found co-occurring in the same crab-hole.

Taxa	Average number of mosquitoes per crabhole	Locality (Province)	Geographic coordinates
Cancer Group ***Deinocerites cancer***	328/59 = 5.6	Bahia Azul (Bocas del Toro)	9.133 N; - 81.85 S
Epitedeus Group ***Deinocerites epitedeus***	179/31 = 5.8	Bahia Azul (Bocas del Toro)	9.133 N; - 81.85 S
Spanius Group *Deinocerites atlanticus*	291/48 = 6.1	Sherman (Colon)	9.365 N; - 79.96 S
Spanius Group *Deinocerites spanius*	213/51 = 4.5	Llano Bonito (Panama)	9.033 N; - 79.45 S
Pseudes Group *Deinocerites pseudes*	290/44 = 6.6	Rio de Jesus (Veraguas)	8.001 N; - 81.17 S
Dyari Group *Deinocerites diary*	117/30 = 4.0	Chame (Panama)	8.601 N; - 79.91 S
Dyari Group *Deinocerites diary*	205/46 = 4.4	Pedasi (Herrera)	7.566 N; - 80.03 S

Females of *De. cancer*, *De. epitedeus,* and *De. pseudes* have been collected with CDC light traps in the past, but some species of *Deinocerites* are repelled by artificial light (Adames 1971). Also, we have gathered good numbers of both female and male *Deinocerites* mosquitoes in Panama, using migration-malaise type of traps (http://johnwhock.com), especially when they were set near areas of mangrove forests. However, these trapping methods cannot be used to investigate patterns of species coexistence, as this can only be tackled by using crabholes as sampling units. O'Meara and Mook (1990) used the operative logic of exit traps, and evaluated two sampling methods for collecting adult *De. cancer* from burrows of *Cardisoma guanhumi* (e.g., Great Atlantic land crab). They flooded the burrow with water or used inverted funnels to capture escaping mosquitoes with a battery-powered aspirator or into collecting bottles, respectively. As stated by the authors, these methods have limitations, as a considerable amount of water is needed to fill the burrows and it is critical to have nearby sources. Also, some mosquitoes would be captured with the aspirator, but others would likely escape. In addition, as land crabs normally exit burrows at sunset, a heavy funnel is needed to prevent the crab from dislodging the exit trap. Our sampling tool has clear advantages over these methods, mostly relative to increasing capture rates and reducing manual labor and sampling biases. It does not involve the difficulties when dealing with water, or setting and revisiting holes at sunset, which significantly reduces sampling effort and bias. Also, escape rates are likely to be lower with our tool because mosquitoes have very few chances to fly away. However, differences in the efficacy of these three methods must be formally tested in future studies.

Our domestic collecting apparatus is simple, economical, and easy to assemble, allowing for the collection of great numbers of *Deinocerites* mosquitoes. This device will be useful to address important ecological questions in this group of arthropods, including crab-mosquito species interactions, community structure, seasonality, and population dynamics. Female *Deinocerites* collected from the same hole can be pooled by species and tested for infection with arboviruses. Moreover, blood-engorged mosquitoes can be used to examine host-feeding patterns in relation to habitat degradation and climate change affecting the transmission cycles of these arboviruses. Our sampling tool has some limitations, though, and could certainly be improved with additional changes. For example, its efficiency may vary depending upon the size and depth of crab holes and may probably work better with shallower and narrower holes as compared to larger and deeper ones. Also, in its original presentation, a person is needed to hold the mosquito trapping cage, which would preclude individual researchers from using it when working on their own. We are certain that these and other details can be further tackled with some creativity, so that the device increases its efficacy (i.e., using a portable electric pump rather than a manual pump, adjusting the length of the air-blowing cylinder to different hole depths, or developing a single-user type of apparatus). Finally, the usefulness of our sampling tool should also be tested to collect resting mosquitoes from tree holes and other such cavities.

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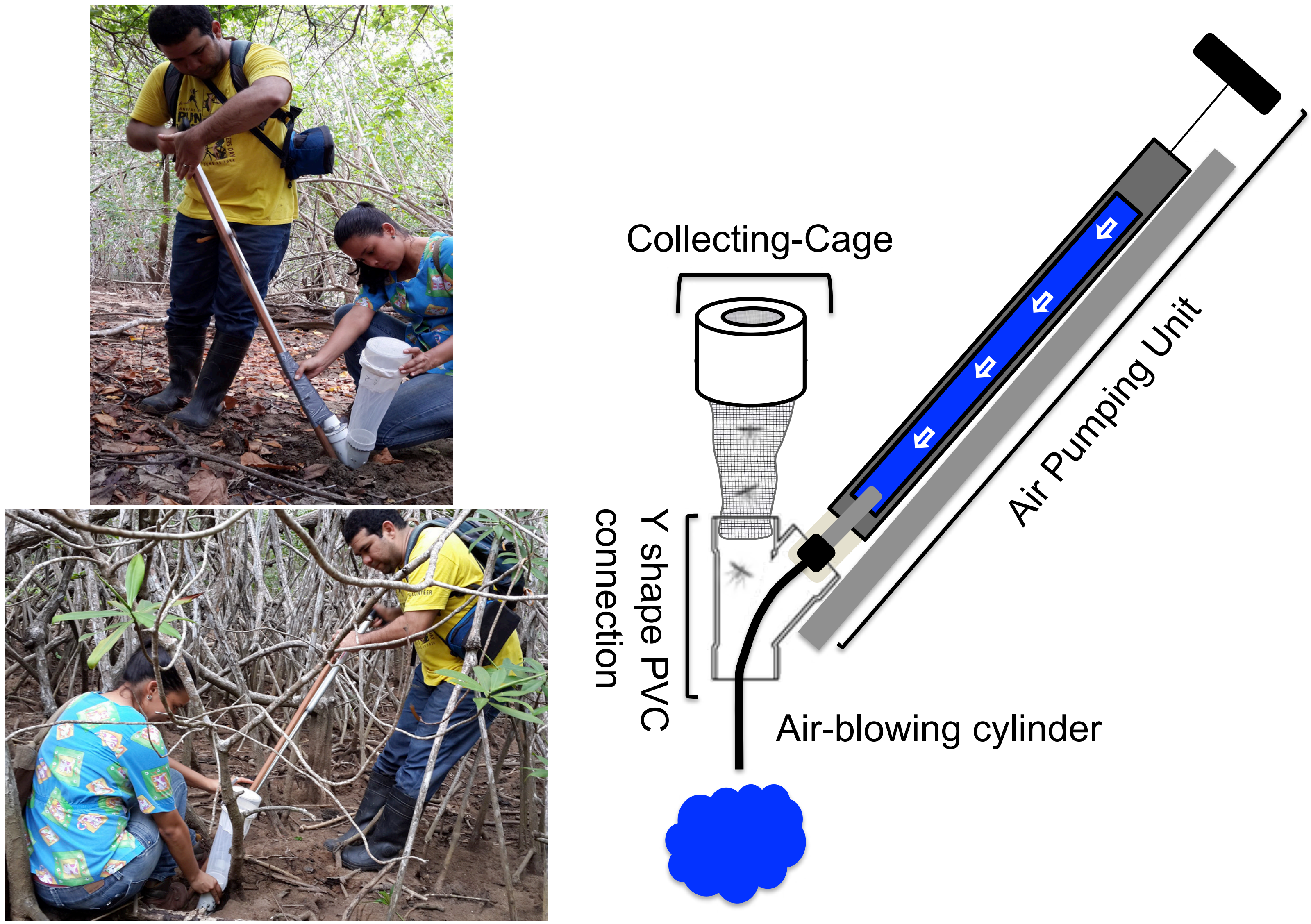

Figure 1. Domestic apparatus used to collect crab-hole inhabiting Deinocerites mosquitoes in Panama. Adult mosquitoes are collected in a cage as they are disturbed and forced to leave the inside of the hole by a flow of pumping air. The air-blowing cylinder (e.g., hosepipe) introduced into the hole is about 40 centimeters (cm) long and roughly 1 cm in diameter.

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