# Performance of light-emitting diode traps for collecting sand flies in entomological surveys in Argentina

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ABSTRACT: The performance of two light-emitting diode traps with white and black light for capturing phlebotomine sand flies, developed by the Argentinean Leishmaniasis Research Network (REDILA-WL and REDILA-BL traps), were compared with the traditional CDC incandescent light trap. Entomological data were obtained from six sand fly surveys conducted in Argentina in different environments. Data analyses were conducted for the presence and the abundance of *Lutzomyia longipalpis*, *Migonemyia migonei,* and *Nyssomyia whitmani* (106 sites). No differences were found in presence/absence among the three types of traps for all sand fly species (p>0.05). The collection mean of *Lu. longipalpis* from the REDILA-BL didn´t differ from the CDC trap means, nor were differences seen between the REDILA-WL and the CDC trap collection means (p>0.05), but collections were larger from the REDILA-BL trap compared to the REDILA-WL trap (p<0.05). For *Mg. migonei* and *Ny. whitmani*, no differences were found among the three types of traps in the number of individuals captured (p>0.05). These results suggest that both REDILA traps could be used as an alternative capture tool to the original CDC trap for surveillance of these species, and that the REDILA-BL will also allow a comparable estimation of the abundance of these flies to the CDC light trap captures. In addition, the REDILA-BL has better performance than the REDILA-WL, at least for *Lu. longipalpis*. ***Journal of Vector Ecology* 40 (2): 373-378. 2015**.

*Keyword Index: Lutzomyia longipalpis, Nyssomyia whitmani, Migonemyia migonei,* light trap, light-emitting diodes, visual targets.

## INTRODUCTION

The battery-operated suction light traps developed by Sudia and Chamberlain (1962), or slightly modified versions, are among the capture tools most used in research for monitoring of Phlebotominae. These traps have some advantages over other types of surveillance traps such as non-light suction traps, animal-baited traps, or those using a carbon dioxide source such as dry ice which increases operational expense and may sublimate at differing rates: A) Standardization: these traps could be used simultaneously without 'bait' heterogeneities (identical light vs bias between different hosts or humans), B) Operational issues: low numbers of technical personnel are required, and it could be left operating overnight automatically, and C) Acceptability and ethical considerations: do not require humans, thus alleviating risk of infection for pathogen-carrying insects and are better accepted by the community as they are less intrusive (indoor manual trapping vs no-person trapping) (Alexander 2000, Cohnstaedt et al. 2008).

However, there are some limitations of the traps for estimating sand flies as an indicator of risk for leishmaniasis. These include light attractiveness; light instead of a host generates bias in species proportion, abundance, sex-rate, physiologic state, and competition with other light sources (moonlight, urban light sources); spatial area of influence: the suction light traps attract sand flies from a lesser distance range, from 2 to 6 m, usually less than 3 m, according to species, placement, and light power; and operational: power consumption and bulkiness of carried batteries and associated costs of replacing expended batteries or having to recharge batteries (Davies et al. 1995, Galati et al. 1997, Alexander 2000, Cohnstaedt et al. 2008, Gaglio et al. 2014, Gebresilassie et al. 2015). To overcome these difficulties, we reviewed available literature on the combination of different trapping methods, a change of trap configuration, and the addition of baits ($CO_2$, kairomones-(octenol)) to compare the effectiveness of these traps against standard CDC light traps (Chaniotis 1983, Wheeler 1996, Andrade et al. 2008, Faiman et al. 2009, Hoel et al. 2011, Kline et al. 2011, Pinto 2011, Obenauer et al. 2012, Kirstein et al. 2013, Signorini et al. 2013, Müller et al. 2015).

Furthermore, the light-trap's narrow radius of attractiveness requires numerous traps for monitoring a given area, and consequently operational costs become an impediment to the use of these traps as a regular methodology for large-area surveillance programs. Therefore, alternative traps were developed (Burkett et al. 1998, Müller et al. 2011, Obenauer et al. 2011), and a change from an incandescent lamp to light-emitting diodes (LEDs) at different wavelengths was evaluated (Hoel et al. 2007, Cohnstaedt et al. 2008).

In this study, we compared a ring light-emitting diode (LED)-based trap (REDILA) with white or black (ultraviolet) light against the traditional CDC-incandescent light

(Miniature Light Trap Model 512 John W Hock Company, Gainesville, FL, U.S.A.). The REDILA trap is made with cheap materials, mostly available at field localities, and is easy to build and repair with minimal skills, so as to avoid the need for excessive traps for sand fly monitoring. We compared the REDILA black and white light traps, as there are reports that *Nyssomyia whitmani* prefers black light Shannon traps while *Migonemyia migonei* prefers white light (Galati et al. 2001, Moschin et al. 2013). In addition, the black or UV lights are usually more productive, mainly with anthropophilic species (Kline et al. 2011, Hashiguchi et al. 2014, Müller et al. 2015). Trap performance was compared in different environmental settings in relation to presence/absence and abundance of *Lutzomyia longipalpis, Mg. migonei,* and *Ny. whitmani*, some of the most important vectors of *Leishmania infantum* and *Leishmania braziliensis* in Argentina (Quintana et al. 2012). Therefore, the aim of this study was to determine whether the REDILA trap, with feasible and affordable characteristics, could be used as an alternative to CDC light traps for surveillance of phlebotomine sand flies.

## MATERIALS AND METHODS

### Trap

The REDILA trap (REDILA 2015, Intellectual Property Registry of Argentina, Figure 1C) consists of one disposable plastic screw-top jar (9 x 19 cm). Two-faced windows were cut in order to allow the light to be seen and attract the insects. To insert the fan (a computer cooler), two additional windows were cut 2.5 cm below the others. Six LEDs were attached to a plastic hair roller, arranged in a crown consisting of two circuits connected in parallel, each circuit composed of three LEDs and a resistor of 120 Ohms (5%, 1.8 W) connected in series. The power consumption of the circuit was 25 mA, and was connected by a wire to a 12 V 7.0 AH battery. The roller was attached at the top of the jar glued to the rubber top to avoid the interference of the plastic pillars with the LED lights (Figure 1A). A rope was also attached to the jar top to hang the trap. The collecting bag was a nylon stocking fixed with rubber bands to the open bottom of the jar (Figure 1B). The stockings could be used as disposable bags, or in case of need for live capture, a light plastic ring could be put inside to keep the bag open.

The Laboratory of Bioelectricity, Faculty of Bio–Engineering, National University of Entre Rios, Argentina, performed the spectral analyses of the lamps and the wavelength (λ) emitted by each one. For the incandescent lamp of the CDC trap, a continuous spectra with a central $\lambda$ = 438 nm (purple) and traces in the near and middle infrared; for the REDILA trap 'white' LED, a central $\lambda$ = 447 nm (purple) and a secondary component in 536 nm (green) with the same spectrum of the incandescent lamp in the visible range; and for the REDILA trap 'black' LED light a central $\lambda$ = 489 nm (blue) with a very low emission in the other sector of the visible spectrum, although it is marked by the manufacturer as $\lambda$ = 390 nm. The maximum sensitivity of *Lu. longipalpis* vision was in the UV region (females $\lambda$ = 520 nm, males $\lambda$ = 546 nm, more related with intensity), with a second peak in the blue-green-yellow region (Mellor et al. 1996).

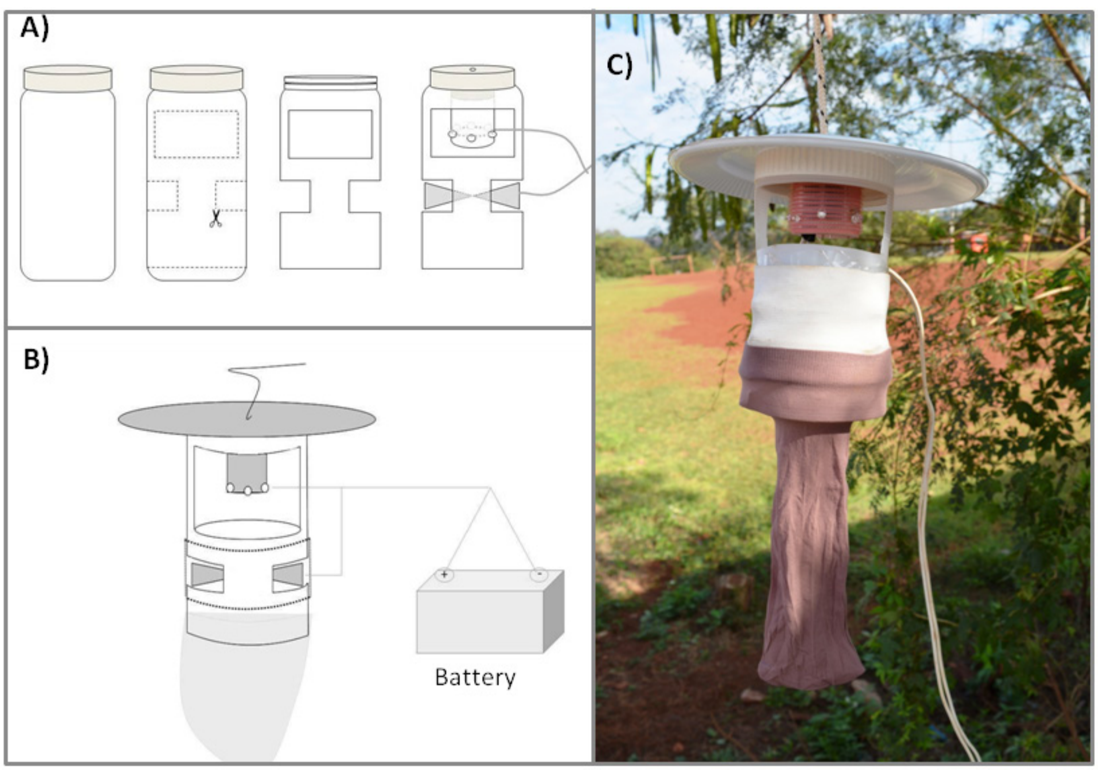


Figure 1. The light-emitting diode traps. (A) First steps in the building of the trap, with cutting the windows in the palstic jar and assembly of the trap. (B) Schematic of the completed trap connected to the battery. (C) Photograph of the finished trap.

Table 1. Description of the six sampling surveys included in the study. Argentina (2010-2014).

| Sampling ID | Date (year-month/s) | Provinces | # sampled sites | Environments | Type of traps used | Positive sites | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Lu.long | Mg.mig | Ny.whi |
| I | 2010-02,03 | Corrientes<br>Entre Ríos | 25 | Urban | Redila-WL<br>Redila-BL | 12 | 2 | 0 |
| II | 2010-02,03 | Corrientes<br>Entre Ríos | 26 | Urban | CDC<br>Redila-WL | 16 | 3 | 0 |
| III | 2011-01,02, and 03 | Entre Ríos<br>Santiago del Estero | 106 | Urban | CDC<br>Redila-BL | 1 | 22 | 0 |
| IV | 2011-04 | Corrientes | 50 | Urban<br>Rural<br>Forest | Redila-WL<br>Redila-BL | 6 | 15 | 3 |
| V | 2011-09 | Misiones | 46 | Urban and periurban | CDC<br>Redila-BL | 14 | 5 | 14 |
| VI | 2013-11<br>2014-01 | Misiones | 21 | Urban<br>Rural<br>Forest | CDC<br>Redila-WL<br>Redila-BL | 7 | 4 | 11 |
| Total | | | 274 | | | 56 | 51 | 28 |

Lu.long=*Lutzomyia longipalpis*, Mg.mig=*Migonemyia migonei*, Ny.whi=*Nyssomyia whitmani*. CDC, miniature light trap Model 512 John W. Hock Company, REDILA-WL, white-light LED trap, REDILA-BL, black-light LED trap.

**Entomological data**

The entomological data were obtained from six entomological surveys conducted in Argentina between 2010 and 2014 in peridomiciles of households, in urban, periurban and rural scenarios, and in forest environments. At each site, two or three trap types were used, thereby generating an incomplete block design (Table 1). One trap per night was used, from approximately 18:00 (sunset) to 09:00 (sunrise). At each site, traps were randomly assigned on successive nights. For each trap, the number of individuals of different sand fly species were recorded. Thus, we compared the presence and abundance of *Lu. longipalpis*, *Mg. migonei,* and *Ny. whitmani* (97% of the overall capture) (Table 1).

**Data analysis**

To analyze the variation in the presence/absence of sand fly species in the three different types of traps, we used generalized linear mixed models (GLMM) with binomial error structure and logit link function (Zuur et al. 2009). The type of trap and the sex of captures were also included as factors and its interaction. To analyze the variation in the number of individuals/species in the three different types of traps, we used a GLMM with a Poisson distribution errors and log as link function. However, the Poisson model showed over-dispersed data, so a negative binomial distribution of the data was assumed for the analysis (Zuur et al. 2009).

In all models, the sampled site was considered as a random factor. The use of GLMM allowed us to overcome the incomplete block design. When the interaction of type of trap-sex was not significant, the sex factor was excluded from the model and the total abundance was used as response variable. Sites without captures were not included in the analysis.

GLMM were conducted using the lme4 (Bates et al. 2013) and MASS packages (Venables and Ripley 2002) from the R software (R Core Team 2013). Tukey multiple comparisons were performed using the multcomp package (Hothorn et al. 2008) from R software.

RESULTS

A total of 7,207 sand flies was captured in 106 sampled sites (45.1% of the total sampled sites) and were included in the analysis. The 4,399 *Lu. longipalpis*, 2,240 *Ny. Whitmani,* and 568 *Mg. migonei*, represented 59.3%, 30.2%, and 7.7% of total captures, respectively. Other species captured, but not included in the data analysis because of their low numbers (<3%), included *Ny. neivai*, *Evandromyia cortellezi/sallesii*, *Pintomyia pessoai, Brumptomyia brumpti, Psathyromyia shannoni, Expapillata firmatoi,* and *Micropygomyia quinquefer.*

*Lutzomyia longipalpis* distribution was mainly urban and periurban but with some occurrences in rural areas. When it was present, it varied from one to 830 individuals per trap-night. *Nyssomyia whitmani* was found in all the three environments, mainly in Misiones province (samplings V and VI, Table 1), and when it was present, the abundance varied from one to 412 individuals per trap-night. There were few records of this species in Corrientes province (three individuals, sampling IV). *Migonemyia migonei* was also present in all sampled environments. The abundance of *Mg. migonei* was lower in relation to the other two species (maximum capture of 103 individuals per trap-night). *Lutzomyia longipalpis* and *Mg. migonei* were recovered from all sampling surveys included in the analysis (Table 1).

For the three species, the interaction type of trap-sex was not significant (p>0.05). No differences were found in presence/absence among the three traps types for all collected species (p>0.05 in all cases, Table 2). The number of *Lu. longipalpis* captured in REDILA-BL and CDC traps was not significantly different, nor were captures different between REDILA-WL and CDC traps. There was a significant difference in collection means between REDILA traps with the black-light trap outperforming the white-light trap (p<0.05, Table 2). For *Mg. migonei* and *Ny. whitmani,* no differences were found among the three types of traps (p>0.05, Table 2).

Table 2. Comparison of presence and abundance by traps for the three sand fly species studied in six entomological studies conducted in Argentina (2010-2014).

| | Sand fly species | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Lu. longipalpis* | | | *Mg. migonei* | | | *Ny. whitmani* | | |
| Number of sites[a] | 56 | | | 51 | | | 28 | | |
| Type of trap | CDC | REDILA-WL | REDILA-BL | CDC | REDILA-WL | REDILA-BL | CDC | REDILA-WL | REDILA-BL |
| Number of traps[b] | 38 | 41 | 40 | 34 | 24 | 48 | 25 | 14 | 28 |
| % Traps with presence (mean, SE) | 81.58, 6.29 | 70.73, 7.11 | 77.50, 6.60 | 67.65, 8.02 | 66.67, 9.62 | 64.58, 6.90 | 72.00, 8.98 | 85.71, 9.35 | 71.43, 8.54 |
| | a | a | a | a | a | a | a | a | a |
| Nº of specimen per trap (mean, SE) | 7.05, 1.33 | 3.91, 1.31 | 10.8, 1.32 | 3.34, 1.27 | 1.82, 1.45 | 2.87, 1.32 | 3.80, 1.46 | 3.62, 1.41 | 2.96, 1.31 |
| | a,b | b | a | a | a | a | a | a | a |

[a]For each species, only sites with at least one specimen captured were analyzed. [b]Because an incomplete block design was applied, the number of traps is different from the numbers of sites and it is different for the three types of traps studied. CDC, miniature light trap Model 512 John W Hock Company, REDILA-WL, white-light LED trap, REDILA-BL, black-light LED trap. Different letters indicate statistically significant differences (p<0.05).

## DISCUSSION

Our results suggest that the REDILA-BL and the REDILA-WL traps could be used as an alternative capture tool to the original CDC light trap to detect the presence of *Lu. longipalpis*, *Mg. migonei,* and *Ny. whitmani* in wide-country surveillance strategies without sacrificing sensitivity in sites, even with a low number of vectors detected by the standard trapping method. The results also indicate that the REDILA-BL allow a comparable estimation of abundance with the CDC-light trap, even in peridomestic urban scenarios, where artificial lights would compete with light 'attractiveness'. In addition, the REDILA-BL has better performance than REDILA-WL, at least for *Lu. longipalpis*, without significant differences for *Ny. whitmani* or *Mg. migonei*. *Nyssomyia whitmani* has shown a preference for the Shannon black traps in Brazil (Galati et al. 2001) and *Mg. migonei* to the white Shannon traps (Moschin et al. 2013).

In Egypt, CDC light traps baited with dry ice were compared to blue, green, and red LED-lights traps. The red-light trap showed a better performance than the CDC white-light trap, collecting more than twice the number of sand flies than the CDC trap (Hoel et al. 2007). Other trap models allow for the selection of LED bulbs of varying colors, viewing angles, or light intensity. One trap using a four-bulb UV-LED light collected 31% more sand flies than the white incandescent CDC trap (French Guiana), while a 16-bulb UV-light collected 42% more than the CDC light trap and 50% more than the trap with a fluorescent UV light bulb (Colombia) (Cohnstaedt et al. 2008).

These results showed the importance of using the same type of trap or combination of traps to generate comparable and consistent entomological data and to avoid biases due to methodology. Traps should be evaluated for each species of interest; the evaluation of these traps for *Ny. neivai*, also a primary vector of *L. braziliensis* in many regions of Argentina, is in progress. When using REDILA traps, is essential to carefully follow the instructions for manufacturing these traps to avoid differences in the efficiency between traps.

In conclusion, the REDILA-BL is an inexpensive and easy-to-make and repair LED-based trap that gives comparable collection performance to the incandescent CDC light trap for sampling sand flies. Thus, it could readily be used as the surveillance trap of choice for the sand fly control program in Argentina. Major difficulties associated with sand fly surveillance include separation of Phlebotomine flies from other insects, clearing, mounting, and identifying the flies to species, and the technical skills and laboratories needed to perform this work. To overcome the last problem, the traps have disposable bags (stockings) that could be sent to the laboratory. Likewise, a reference entomologist and laboratory are required to identify the sand flies at the species level.

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