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Original Paper

Sand Fly Fauna (Diptera, Pcychodidae, Phlebotominae) in Different Leishmaniasis-Endemic Areas of Ecuador, Surveyed Using a Newly Named Mini-Shannon Trap

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Abstract: To study the sand fly fauna, surveys were performed at four different leishmaniasis-endemic sites in Ecuador from February 2013 to April 2014. A modified and simplified version of the conventional Shannon trap was named "mini-Shannon trap" and put to multiple uses at the different study sites in limited, forested and narrow spaces. The mini-Shannon, CDC light trap and protected human landing method were employed for sand fly collection. The species identification of sand flies was performed mainly based on the morphology of spermathecae and cibarium, after dissection of fresh samples. In this study, therefore, only female samples were used for analysis. A total of 1,480 female sand flies belonging to 25 *Lutzomyia* species were collected. The number of female sand flies collected was 417 (28.2%) using the mini-Shannon trap, 259 (17.5%) using the CDC light trap and 804 (54.3%) by human landing. The total number of sand flies per trap collected by the different methods was markedly affected by the study site, probably because of the various composition of species at each locality. Furthermore, as an additional study, the attraction of sand flies to mini-Shannon traps powered with LED white-light and LED black-light was investigated preliminarily, together with the CDC light trap and human landing. As a result, a total of 426 sand flies of nine *Lutzomyia* species, including seven man-biting and two non-biting species, were collected during three capture trials in May and June 2014 in an area endemic for leishmaniasis (La Ventura). The black-light proved relatively superior to the white-light with regard to capture numbers, but no significant statistical difference was observed between the two traps.

Key words: sand fly fauna, *Lutzomyia* spp., a newly named mini-Shannon trap, CDC light trap, black- and white-light trap, Ecuador

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Introduction

Several species of sand flies (Diptera, Psychodidae, Phlebotominae) transmit agents of vector-bone diseases such as leishmaniasis, bartonellosis, phleboviruses, orbiviruses, vesiculoviruses and saurian malaria [1–3]. With regard to leishmaniasis, the parasites of the genus *Leishmania* are transmitted by the bite of female sand flies of the genus *Phlebotomus* in the Old World and the genus *Lutzomyia* in the New World. Approximately 800 sand fly species have been recorded, but less than 10% of them transmit each particular *Leishmania* species [4–7]. Studies on sand fly species are therefore an important aspect of risk assessment for diseases in leishmaniasis-endemic areas. In this context, almost all attempts to study sand fly species and their behavior as part of the surveillance and/or control of the vectors involve intensive population sampling [8, 9].

For studies on leishmaniasis transmission, the collection of adult phlebotomine sand flies is essential in endemic areas, and several standard techniques, such as protected human landing, CDC light trap, Shannon light trap and sticky paper collections have been implemented for that purpose [8–10]. Among the above, human landing collection is the most convenient way for researchers and public health personnel to obtain information on man-biting behavior of sand fly species in given areas. This technique, however, involves the risk of the collectors becoming infected with *Leishmania*. An important prerequisite for the estimation of the intensity of leishmaniasis transmission is the calibration of the sampling methods used against the protected human bait catches. This is because protected human bait catches translate directly into "human biting rates", which serve as an essential parameter in the estimation of both entomologic inoculation rate and vectorial capacity [10]. Among the remaining collection methods, i. e. CDC, Shannon light traps and sticky paper traps, the former two are widely used standard surveillance capture methods, while the latter one is usually employed for taxonomic investigations of sand flies. Many kinds of sand fly collection methods and traps were reviewed previously [8, 9].

We used to employ the conventional Shannon light trap [11] for sand fly collection in leishmaniasis-endemic areas. To set the Shannon trap, however, it is necessary to procure a considerably large space because of the cumbersome cover-tent. As an improvement, we modified the conventional Shannon trap for use in narrow and limited spaces in forested or mountainous areas and named it the mini-Shannon trap.

Here, we report a study on the fauna of phlebotomine sand flies in leishmaniasis-endemic areas of Ecuador employing the mini-Shannon trap, CDC light trap and protected human landing collection. Another aim of this study was to determine whether the mini-Shannon trap can be used in place of other methods such as the conventional Shannon trap, CDC light trap and protected human landing collection to estimate or monitor available sand fly species in a given area. The sand fly samplings were conducted and evaluated in different leishmaniasis-endemic areas of Ecuador.

Methods

Study sites

Ecuador is a highly diverse country with marked geographic, climatic, ecologic and pathologic differences between each natural region. The Andes traverse Ecuador from north to south and divide the country into three natural regions: the Pacific coast and the Andean and Amazonian regions. There are 24 provinces including the Galapagos Islands. Of them, 21 provinces have recorded *Lutzomyia* sand flies and *Leishmania* parasites. The present study was conducted at the following four localities in Ecuador from March 2013 to April 2014 (Fig. 1): 1)

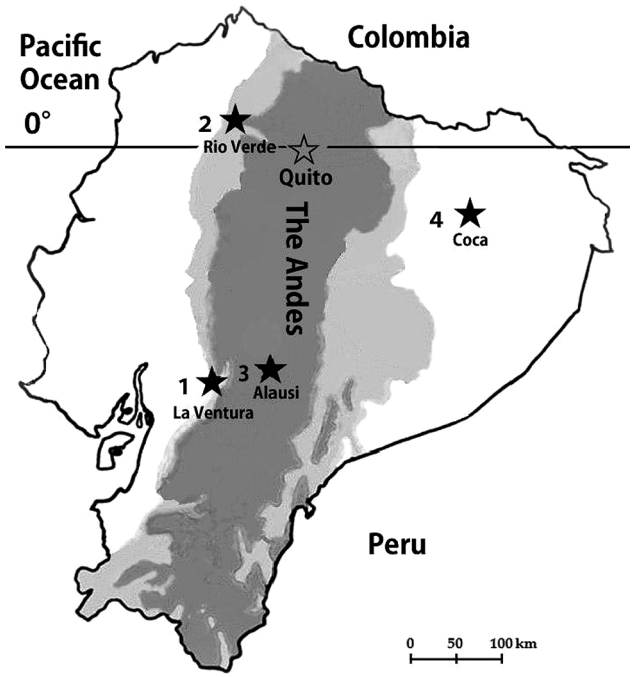

Fig. 1. Map of Ecuador, showing the four study sites: 1. La Ventura-Cumanda, Province of Chimborazo (300 m above sea level), 2. Rio/Cielo Verde, Province of Imbabura (600 m asl), 3. Alausi, Province of Chimborazo (2300 m asl), 4. Coca (Puerto Francisco de Orellana), Province of Orellana (240 m asl).

La Ventura (300 m above sea level [a.s.l.]), Province of Chimborazo on the Pacific coast, 2) Rio/Cielo Verde (600 m a.s.l.), Province of Imbabura on the Andean slope (Cordillera), 3) Alausi (2300 m a.s.l.), Province of Chimborazo in the Andes, and 4) Coca (Puerto Francisco de Orellana) (240 m a.s.l.), Province of Orellana in the Amazonian region. For the comparison of the attraction of sand flies to LED white-light or LED black-light, additional capture trials were conducted during May-June 2014 at one of the study sites (site 1: La Ventura) using the mini-Shannon trap.

Sand fly collection

Two light traps, i.e. the newly named mini-Shannon trap and a commercial CDC light trap, were used for the sampling of sand flies in addition to human landing collection.

The newly named mini-Shannon trap

Basically, the mini-Shannon trap was made from the plastic frame of a washing hanger and a light cotton cloth-cover tent instead of netting, as shown in Fig. 2. The cloth-cover tent was handmade to fit each size and dimension (Fig. 2a), and the cloth-cover was fitted to the plastic hanger (Fig. 2b) during the setting of the trap in the field. The light (LED white- and black-light) was suspended from the top-inside of the cloth-cover tent fitted to the plastic hanger frame. The lower part of the tent can be closed with a string to prevent the invasion of insects including sand flies and other untargeted insects (Fig. 3). The total weight of the trap is only about 300 g, making it very easy to transport.

CDC light trap

A commercial miniature CDC light trap equipped with an incandescent light (John Hock, Gainesville Florida, U.S.A., model 512) was used.

Protected human landing collection

Protected human landing (authors participated) was employed to study the composition of man-biting sand fly species at each site.

Sampling of sand flies

On each collection trial, the mini-Shannon trap was set with CDC light traps suspended beside it, and some of the authors conducted a human landing catch at the same place in the four study areas (sites 1–4 in Fig. 1). The mini-Shannon and the CDC light traps were set about one meter apart from tree branches or wire 30–40 cm above the floor. Samplings were performed simultaneously from 18:00 to 21:00 each night (one to three visits depending on the study site) from February 2013 to April 2014. Furthermore, as a preliminary study, attractiveness tests were also conducted using the mini-Shannon traps powered with LED white- and LED black-lights, together with CDC light traps and human landing, from May to June 2014 at the collection site 1 (Fig. 1). The light traps were installed in the nearby forest, at least 100–200 m from the houses in the study areas. After each trap was set (18:00), the collector aspirated the insects attracted to the light from the wall of the cloth-cover and/or the upper part of the mini-Shannon trap, using an insect-aspirator. Most of the insects including sand flies gathered in the upper part of the trap. Human landing sand flies were also captured using the aspirator, close to the light traps set at the same place.

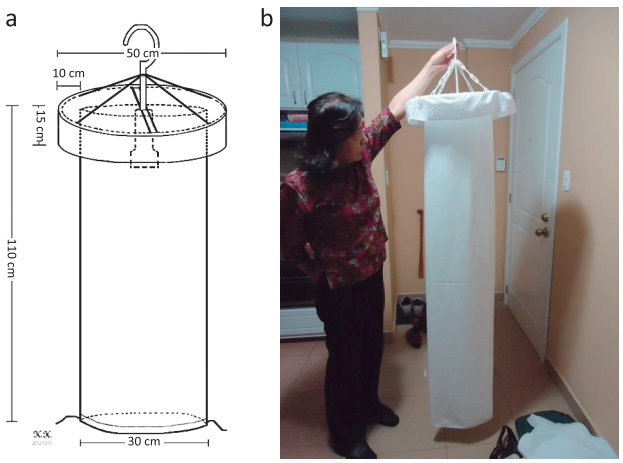

Fig. 2. a. A modified and simplified schematic mini-Shannon trap, with the dimensions of each part, equipped with a light at the top-inside. b. A homemade, mini-Shannon trap.

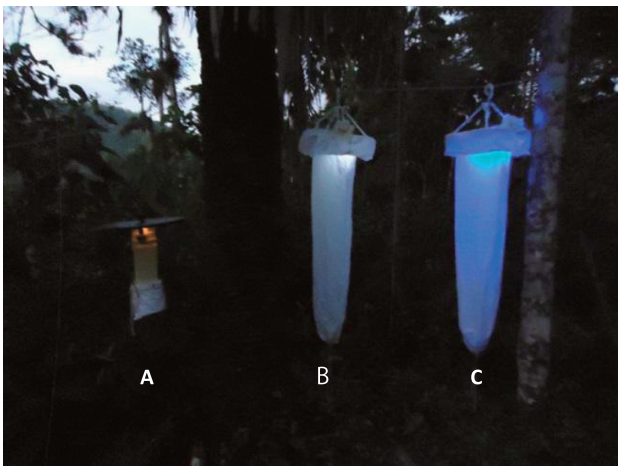

Fig. 3. Collocated CDC light trap (A) and mini-Shannon traps equipped with LED white-light (B) and LED black-light (C) at the collection site of La Ventura, Chimborazo, Ecuador.

Processing of the collected sand flies

The sand flies were taken to the field laboratory and killed either by freezing or by suffocation with 70% ethanol vapor. Male and female sand flies were separated and counted. All the female sand flies collected were dissected by the method described previously [12], and the specimens were identified mainly based on the morphology of spermathecae, cibarium and taxonomic keys proposed by Young and Duncan [13]. At the same time, natural infection of sand flies with *Leishmania* and *Endotrypanum* promastigotes was also determined by examining the gut content under a microscope. These results will be published elsewhere. After counting, all the male specimens were kept in 100% ethanol until further processing, without identification. In this study, therefore, only the female samples identified at the species level were used for data analysis.

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 17.0 (Armonk, NY: IBM Corp). The abundance data of collected sand flies did not meet the normal distribution assumption. Therefore, to assess differences among traps and numbers of sand flies captured, non-parametric contrast tests (Kruskal-Wallis and Mann-Whitney) were applied in this study.

Results

Sand fly species collected at the four localities

In this study, a total of 1,480 female specimens belonging to 27 *Lutzomyia* species were collected (Table 1). The number of female sand flies collected in each trap was 417 by mini-Shannon, 259 by CDC light trap and 804 by human landing. The total number of sand flies collected by the different methods was affected by the collection site, i.e. Pacific coast (site 1), Andean slope (site 2), Andes (site 3) and Amazon (site 4) (Fig. 1), mainly because of the different species composition. Therefore, the capture numbers are not suitable for direct comparison, because of the different capture trials at different study sites, and no statistical analysis was performed on the data shown in Table 1. For the same reason, no precise calculation was done on the index of species richness and diversity. However, it is noteworthy that the number of sand fly species collected by the three methods i.e. mini-Shannon, CDC light trap and human landing, varied from 1 to 14 species per site tested (Table 1). At site 1, *Lu. panamensis* was the most frequent species captured by the three methods, followed by *Lu. trapidoi* or *Lu. hartmanni*. At site 2 it was *Lu. panamensis* followed by *Lu. aclydifera* or *Lu. trapidoi*. At site 3 only 1 species, *Lu. ayacuchensis*, was available, and at site 4 it was *Lu. yuilli yuilli* followed by *Lu. tortura.*

Protected human landing collection produced excellent results at every collection site, except site 4 located in the Amazon where *Lu. yuilli yuilli* showed an extremely high attraction to mini-Shannon (LED white-light) trap, accounting for 162 (70.1%) of the 231 flies collected. On the other hand, almost all the *Lu. tortura* specimens were collected by human landing at that study site, only one sample being collected by LED white-light trap and nil by CDC light trap. At study site 3 (Alausi) located at the Andes where *Lu. ayacuchensis* is prevalent, 349 (90.65) of the total 385 flies collected by the three methods were captured by human landing, suggesting the extremely high anthropophilic behavior of this highland sand fly species. In the Andes area, the CDC light trap tended to attract relatively higher numbers (29 flies) of *Lu. ayacuchensis* than the mini-Shannon (LED white-light) trap (7 flies).

In the present study, the following 15 of the total 25 *Lutzomyia* species were collected by protected human landing: *Lu. gomezi*, *Lu. robusta*, *Lu. panamensis*, *Lu. shannoni*, *Lu. hartmanni*, *Lu. trapidoi*, *Lu. maranonensis*, *Lu. ayacuchensis*, *Lu. tortura*, *Lu. flaviscutelata*, *Lu. carrerai carrerai*, *Lu. yuilli yuilli*, *Lu. hirsuta hirsuta*, *Lu. amazonensis* and *Lu. aclydifera*. In the samplings of principal man-biting species from the Pacific coastal and the Andean slope (Cordillera) (collection sites 1 and 2), the protected human landing method caught 33.3% (10/30), 45.0% (150/333), 56.4% (92/163) and 80% (84/105) of the *Lu. gomezi*, *Lu. panamensis*, *Lu. trapidoi* and *Lu. hartmanni*, respectively, also caught in mini-Shannon and CDC light traps. For the second and third species, i.e. *Lu. panamensis* and *Lu. trapidoi*, there was a relatively proportional sampling between the light traps (mini-Shannon and CDC) and human landing collection, whereas the last species, *Lu. hartmanni*, showed a low attraction to the mini-Shannon (white-light) trap (Table 1). In Coca (site 4), the following five un-identified species were also collected: *Lutzomyia* sp. A, 1 fly; *Lu.* sp. B, 5; *Lu.* sp. C, 1; *Lu.* sp. D, 2; and *Lu.* sp. E,1 by mini-Shannon trap and *Lu.* sp. B, 1 fly by CDC light trap.

The attractiveness of the different collection methods to sand flies

The attraction of sand flies to LED white-light and LED black-light, as well as CDC light trap and human landing, was compared based on the capture numbers of the three tests performed preliminarily at site 1 (La Ventura) arranged according to *Lutzomyia* species as shown in Table 2. In this trial, a total of 426 sand flies of nine *Lutzomyia* species was collected and recorded, includ-

Table 1. Capture numbers/trap of sand flies collected by different methods at four sites (1–4) of Ecuador, arranged by 25 *Lutzomyia* species (February 2013–April 2014).

Lutzomyia spp.	Collection methods and collection sites (1*, 2**, 3***, 4****)												Total
	Mini-Shannon (LED[#]) trap				CDC light trap				Human landing collection				
	1	2	3	4	1	2	3	4	1	2	3	4	
gomezi	10			1	7	1			10			1	30
robsuta	1	1			3				5			2	12
panamensis	92	32			30	29			78	72			333
shannoni	9				6				2				17
hartmanni	2	3			12	4			67	17			105
trapidoi	32	3			36				65	27			163
maranonensis	1			2	3				2				8
aclydifera		6				17			1				24
sanguinaria		1											1
barretoi majuscula		6				3							9
bifoliata		1											1
dysponeta	4					1							5
camposi		1				2							3
reburra		3				2							5
aragaoi		4				5							9
ylephiletor		3				4							7
triramura		2				1							3
sordellii		2				1							3
ayacuchensis			7				29				349		385
tortura				1								67	68
flaviscutellata												9	9
carrerai carrerai				1				1				9	11
yuilli yuilli				162				53				16	231
hirsuta hirsuta				21				6				3	30
amazonensis				1								2	3
Total	151	70	7	189	97	73	29	60	230	116	349	109	1,480
Total no. of species/site	8	14	1	7	7	12	1	3	8	3	1	8	

*1. La Ventura (3 trials: during 18:00–21:00). **2. Rio/Cielo Verde (3 trials: 18:00–21:00). ***3. Alausi (3 trials: 17:00–20:00). ****4. Coca (One trial: 18:00–21:00); a part of data, shown by Kato et al. [14]. #, LED White-light.

ing seven man-biting species and two non-biting species. Again, human landing collection was superior with regard to capture numbers, followed by the CDC light, black-light and white-light traps. However, when the capture efficiency of each trap was compared based on the data shown in Table 2, the only statistically significant difference was that found between human landing and both white- and black-light traps. It is noteworthy that no significant differences were observed between any other pairs, even between the mini-Shannon traps equipped with white- and black-lights, or between the CDC light trap and the mini-Shannon traps (Table 3).

Among the nine man-biting species, *Lu. trapidoi* (199, 46.7%) was the most abundant in this area, followed by *Lu. panamensis* (114, 26.8%), *Lu. hartmanni* (48, 11.3%) and *Lu. gomezi* (29, 6.8%). *Lutzomyia trapidoi* and *Lu. panamensis* seemed to be attracted to the light traps (CDC and both LED white- and black-lights). The capture numbers of the nine *Lutzomyia* species (Table 2) collected were analyzed among traps. The results are shown in Table 4. Significant differences among traps were only found for *Lu. gomezi*, *Lu. hartmanni* and *Lu. trapidoi*. The main difference was the presence of human landing in the analysis; without it, no significant difference was observed in the collection of any species of *Lutzomyia* with either the CDC light trap or both the mini-Shannon white- and black-light

Table 2. The attractiveness test of sand flies using different collection methods, shown by total capture numbers of 3 trials (with range/trap) (May–June 2014).

Lutzomyia spp.	Mini-Shannon LED light trap		CDC light trap	Human landing collection	Total
	White-light	Black-light			
gomezi	2 (0–2)	0	6 (1–3)	21 (5–9)	29
robusta	0	0	1 (0–1)	3 (0–2)	4
panamensis	22 (4–14)	28 (4–12)	32 (7–15)	32 (8–13)	114
shannoni	0	1 (0–1)	0	7 (0–5)	8
hartmanni	2 (0–2)	1 (0–1)	9 (2–4)	36 (11–13)	48
trapidoi	20 (4–10)	27 (8–10)	67 (20–25)	85 (22–32)	199
maranonensis	2 (0–2)	0	2 (0–2)	9 (0–5)	13
camposi	0	3 (0–3)	2 (0–2)	0	5
dysponeta	0	1 (0–1)	5 (0–3)	0	6
Total	48	61	124	193	426

Table 3. Statistically significant differences between pair of traps (LED white-light mini-Shannon, LED black-light mini-Shannon, CDC traps and human landing collection) used in the capture tests, based on non-parametric Mann-Whitney tests.

Pair of traps compared	Mann-Whitney	*p*-value
White vs Black mini-Shannon traps	351.00	0.784
White mini-Shannon vs CDC trap	261.50	0.054
White mini-Shannon vs human landing	210.15	0.004*
Black mini-Shannon vs CDC trap	269.50	0.079
Black mini-Shannon vs human landing	227.50	0.012*
CDC trap vs human landing	300.50	0.257

*Significant.

Table 4. Sand fly species collected and their relationship with the traps used in the capture trial with the Kruskal-Wallis test, expressed in chi-square and *p*-value.

Lutzomyia spp.	Chi square	*p*-value
gomezi	9.34	0.025*
robusta	4.67	0.196
panamensis	0.86	0.830
shannoni	4.90	0.172
hartmanni	9.40	0.024*
trapidoi	9.04	0.029*
maranonensis	3.72	0.029
campos	2.12	0.530
dysponeta	4.90	0.172

*Significant.

traps. Thus, when the data (shown in Table 2) were analyzed, without considering human landing collection, the results revealed no significant statistical difference. The following Chi-square and *p*-values were noted for each species: *Lu. gomezi*, 5.091, 0.078; *Lu. robusta*, 2.000, 0.368; *Lu. panamensis*, 0.835, 0.659; *Lu. shannoni*, 2.000, 0.368; *Lu. hartmanni*, 5.358, 0.069; *Lu. trapidoi*, 5.804, 0.055; *Lu. maranonensis*, 1.143, 0.565; *Lu. camposi*, 1.167, 0.558; and *Lu. dysponeta*, 3.231, 0.199.

Discussion

The two types of light trap, i.e. mini-Shannon and CDC light traps, collected a relatively small number of *Lutzomyia* species. Among the 25 sand fly species shown in Table 1, two species (*Lu. bifoliata* and *Lu. sanguinaria*) captured in Rio/Cielo Verde (site 2) using the mini-Shannon trap were registered for the first time in Ecuador. *Lutzomyia bifoliata*, an anthropophilic species, has only been reported from different localities of the neighboring country of Colombia [13]. Among the samples, *Lu. ylephiletor* was recently recorded in the neighboring province of Pichincha [15]. This species was also reported from Colombia [16], and has already been incriminated as a vector of *L. (L.) mexicana* parasites in Guatemala [17]. Interestingly, many sand fly species were collected by light traps in our collection site 2, i.e. Rio/Cielo Verde, province of Imbabura, indicating an abundant species composition of the genus *Lutzomyia* in that area. In the neighboring province (Pichincha), many species have also been repor-

ted by other workers employing different collection methods: Young and Rogers [18] listed 13 spp.; Alexander et al. [19], 9 spp.; Le Pont et al. [20], 19 spp.; Jones et al. [21], 15 spp.; Zapata et al. [22], 8 spp. More recently, Gomez et al. [15] reported 18 man-biting species in total from the province of Pichincha, Ecuador. We also collected *Lutzomyia aclydifera* using the human landing method at our study site 2 (Rio/Cielo Verde), but the man-biting behavior of this sand fly species should still be investigated in the future [15].

Several methods have been reported and evaluated for sand fly collection with varying degrees of efficiency as reviewed by Alexander [8] and Alexander and Maroli [9]. In the case of incrimination and determination of *Leishmania*-vector species of the genus *Lutzomyia*, human landing collection is an essential and important method. This procedure however puts the participants themselves at a risk of infection with targeted vector/insect-borne diseases. On the other hand, light trap collection methods can provide an estimate of circulating sand fly species at a given endemic site of leishmaniasis, including human and animal biting species. In this study, therefore, we implemented a newly developed mini-Shannon trap, because of the limitations faced by the conventional Shannon trap [11] in narrow, forested and rocky spaces. The mini-Shannon trap was validated by comparison with CDC light trap and protected human landing collection, performed concurrently at four different localities (Fig. 1) of Ecuador endemic for leishmaniasis. The merits of the mini-Shannon trap include the fact that it is: 1) useful for comparative attractiveness tests on sand flies and other targeted insects because of its small and simple design and alternative light-color, light-waves, etc.; 2) made of a very light plastic washing hanger frame and cotton cloth-cover (only 300 g); 3) folding/collapsible mechanism; 4) easy to handle and transport; 5) easy to set up at any place including extremely narrow, forested and rocky areas; 6) easy to change the hanging test place during sand fly capture at night, and 7) inexpensive, costing less than US$20 including LED white- or black-light per trap and it will cost less than US$10 if a normal light is used.

The present mini-Shannon (LED white-light) trap showed a varying efficiency depending on the species of genus *Lutzomyia* circulating at the site tested (Table 1). For example, in the Andes (Alausi, site 3) where only *Lu. ayacuchensis*, a highly anthropophilic species was available [6, 7, 14, 15], an extremely small number of the species was captured using the trap. On the other hand, in the Amazon (Coca, site 4) the trap caught 67.3% of the 211 *Lu. yuilli yuilli* captured, followed by CDC light trap (25.1%) and human landing (7.6%), suggesting a high attraction to the LED white-light. In the Pacific areas (La Ventura and Rio/Cielo Verde, sites 1 and 2), several man-biting species were available. Among them, *Lu. panamensis* revealed a high attraction to both human and light traps, the capture rate being 37.2% (124/333), 45.1% (150/333) and 17.7% (59/333) using the mini-Shannon, human landing and CDC light trap, respectively. The remaining species also seemed to respond well to both human and light traps, though *Lu. hartmanni* (80%: 84/105) showed a preference for humans, followed by *Lu trapidoi* (56.4%, 92/163). Regarding *Lu. tortura* from the Amazon (site 4), almost all of the specimens were captured by human landing collection, only one specimen being captured using our mini-Shannon trap and nil using the CDC light trap at the study site. These results suggested that the employment of both protected human bait and light traps is important for obtaining detailed and exact information on species composition, abundance and the distribution of sand flies circulating at a given study site.

A preliminary test of the attraction of sand flies to light traps was performed in an area endemic for leishmaniasis, i.e. La Ventura (site 1 in Fig. 1), Chimborazo, employing our mini-Shannon traps powered with LED white-light and LED black-light, as well as CDC light trap and human landing. A total of nine *Lutzomyia* species including seven man-biting and two non-biting species were recognized in the study area. The capture result showed that the black-light was relatively superior to the white-light, but no statistically significant difference was observed between the two traps (Table 3). In the attractiveness test, however, the distance between each light trap (one meter) might be insufficient to rule out the interference of one trap over another. Therefore, more detailed test-trials should be performed in future studies, with reference to the distance between each trap.

In conclusion, the total number of sand flies per trap collected by the different methods was markedly affected by the study site, probably because of the various composition of species at each locality tested. The newly named mini-Shannon trap powered with LED white- or black-light caught sand flies in narrow and forested sampling sites, but the trap was not as sensitive as the human landing collection method (statistically significant) or CDC light trap (not statistically significant). However, it promises to be useful for different types of attractiveness test on vector insects where a sufficient distance must be maintained between the traps tested, because of its small size, easy handling and convenient transportation in the field. The mini-Shannon trap may also help health personnel in endemic areas to collect sand fly samples for surveillance at a cost less than commercial CDC light traps. More stud-

ies should be conducted using the mini-Shannon trap to shed light on sand fly behavior and obtain insights for future research and vector control.

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Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Comer JA, Tesh RB. Phlebotomine sand flies as vectors of vesiculoviruses: a review. Parasitologia 1991; 33: 143–150.
2. Ashford RW. *Phlebotomus* fevers. The Encyclopedia of Arthropod-Transmitted Infections. In: Service MW, ed. Wallingford, UK: CABI Publishing, 2001. pp. 397–401.
3. Hughes GL, Samuel SK, Shaikh K, et al. Discrimination of the *Plasmodium mexicanum* vectors *Lutzomyia stewarti* and *Lutzomyia vexator* by a PCR-RFLP assay and *Wolbachia* infection. J Vector Ecol 2014; 39: 224–227.
4. Munstermann LE. Phlebotomine sand flies, the Psychodidae. In: Marquardt WC, Black WC, Freier JE, et al., eds. Biology of Disease Vectors. 2nd ed. San Diego: Elsevier; 2004. pp. 141–151.
5. Bates PA. Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. Int J Parasitol 2007; 37: 1097–1106.
6. Kato H, Gomez EAL, Cáceres AG, et al. Molecular epidemiology for vector research on leishmaniasis. Int J Environ Res Public Health 2010; 7: 814–826.
7. Gomez EAL, Kato H, Mimori T, et al. Distribution of *Lutzomyia ayacuchensis*, the vector of Andean-type cutaneous leishmaniasis, at different altitudes on the Andean slope of Ecuador. Act Trop 2014; 137: 118–122.
8. Alexander JB. Sampling methods for phlebotomine sand flies. Med Vet Entomol 2000; 14: 109–122.
9. Alexander JB, Maroli M. Control of phlebotomine sandflies. Med Vet Entomol 2003; 17: 1–18.
10. Killick-Kendrick R. Methods for the study of phlebotomine sandflies. In: Peters W, Killick-Kendrick R, eds. The Leishmaniases in Biology and Medicine. London: Academic Press; 1987; 1. pp. 473–497.
11. Shannon RC. Methods for collecting and feeding mosquitoes in jungle yellow fever studies. Am J Trop Med Hyg 1939; 19: 131–148.
12. Hashiguchi Y, Gomez EAL, Coronel VV, et al. Natural infections with promastigotes in man-biting species of sandflies in leishmaniasis-endemic areas in Ecuador. Am J Trop Med Hyg 1985; 34: 440–446.
13. Young DG, Duncan MA. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae), Memoirs of the American Entomological Institute, Associated Publishers. Gainesville, FL: Am Entomol Inst; 1994. 54: 1–881.
14. Kato H, Calvopiña M, Criollo H, et al. First human cases of *Leishmania (Viannia) naiffi* infection in Ecuador and identification of its suspected vector species. Acta Trop 2013; 128: 710–713.
15. Gomez EAL, Kato H, Hashiguchi Y. Man-biting sand fly species and the natural infection with *Leishmania* promastigote in leishmaniasis-endemic areas of Ecuador. Act Trop 2014; 140C: 41–49.
16. Young DG. A review of the bloodsucking psychodid flies of Colombia (Diptera: Phlebotominae and Sycoracinae). Gainesville: Tech Bull 806, Agric Exp Station, IFAS, Univ Florida; 1979. pp. 1–226.
17. Porter CH, Steurer FJ, Kreutzer RD. Isolation of *Leishmania mexicana mexicana* from *Lutzomyia ylephiletor* in Guatemala. Trans R Soc Trop Med Hyg 1987; 81: 929–930.
18. Young DJ, Rogers TE. The phlebotomine sand fly fauna (Diptera: Psychodidae) of Ecuador. J Med Entomol 1984; 21: 597–611.
19. Alexander JB, Takaoka H, Eshita Y, et al. New records of phlebbotomine sand flies (Diptera: Psychodidae) from Ecuador. Mem Inst Oswardo Cruz, 1992; 87: 123–130.
20. Le Pont F, Leon R, Mouchet J, et al. Leishmaniose en Equateur. 2. Contactos home/vecteurs de leishmaniose: cas de *Lutzomyia trapidoi* et *Lu. gomezi*. Ann Soc Belge Med Trop 1994; 74: 13–21.
21. Jones LA, Cohnstaedt LW, Beat L, et al. New records of phlebotomine sand flies (Diptera: Psychodidae) from Ecuador. Proc Entomol Soc Wash 2010; 112: 47–53.
22. Zapata S, Mejia L, Le Pont F, et al. A study of a population of *Nyssomyia trapidoi* (Diptera: Psychodidae) caught on the pacific coast of Ecuador. Parasites & Vectors 2012; 5: 144.