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COMPARATIVE EVALUATION OF TRAPPING METHODS FOR PHLEBOTOMINE SAND FLIES IN ROMANIA

Bianca-Lavinia ANDRONIC[1]*, Larisa-Maria IVĂNESCU [1], Gabriela-Victoria APOPEI[1], Raluca MÎNDRU[1], Liviu MIRON[1]

e-mail: lavinia.andronic@iuls.ro

Abstract
Phlebotomine sand flies (Diptera: Psychodidae) are vectors of *Leishmania infantum*, the etiological agent of leishmaniosis, a major vector-borne zoonosis. Although several autochthonous and imported cases have recently been reported in Romania, updated information on sand fly distribution remains limited, highlighting the need for continuous surveillance and reliable trapping tools.
Between May and September of 2023–2025, 55 sand flies were collected from 13 sites in northeastern and southeastern Romania using three trap types: the New Standard Miniature CDC Light Trap (John W.Hock Company, USA), Laika Trap 4.0 (LaikaLab, Pozzuolo, Italy), and sticky traps. Traps were placed from dusk until dawn in suitable microhabitats, and specimens were morphologically identified using standard taxonomic keys.
Light-based traps (CDC and Laika) yielded comparable numbers and accounted for over 95% of all captures, whereas sticky traps were less efficient. Despite technical differences, both light traps performed similarly under field conditions. These findings indicate that light-based traps are effective for routine surveillance, supporting their use in systematic sand fly monitoring programs in Romania.

Key words: sand fly, trapping methods, CDC Light Trap, Laika Trap, sticky trap

Introduction

Phlebotomine sand flies are small, nocturnal, hematophagous insects belonging to the Order *Diptera*, Family *Psychodidae* (Munstermann L.E., 2019). Adults typically measure less than 5 mm and have a yellowish-brown coloration, with a distinctive humped and hirsute appearance. They have long legs and lanceolate wings, which are characteristically held in a „V" shape while at rest (Cosoroabă I., 2000).

Globally, more than 800 recognized species have been described, divided between the Old World (Europe, Asia, Africa) and New World (the Americas). Within the subfamily *Phlebotominae*, six genera are acknowledged (Akhoundi M. *et al.,* 2016): *Phlebotomus*, *Sergentomyia*, and *Chinius* in the Old World, and *Lutzomyia*, *Brumptomyia*, and *Warileya* in the New World.

From a veterinary and public health perspective, sand flies are the exclusive biological proven vectors of *Leishmania* spp. (Order *Kinetoplastida*, Family *Trypanosomatidae*), the protozoan parasites responsible for human and canine leishmaniosis. Furthermore, sand flies also transmit other pathogens of relevant public health importance, such as *Bartonella baciliformis*, the etiological agent of Carrion's disease in South America, and Toscana virus (TOSV), a neuroinvasive phlebovirus associated with human meningitis and encephalitis in Mediterranean countries, particularly in Italy (Maroli M.D. *et al.,* 2013; Andronic B.L. *et al.,*2024). These pathogens collectively underline the broad medical significane of phlebotomine sand flies and the need for vector surveillance programs implementation.

Over the past decade, Europe have witnessed a notable northward expansion of sand flies and sand fly-borne diseases. Climatic warming, environmental change, urbanization, and increased movement of humans and dogs have facilitated the establishment of sand fly species in regions previously considered unsuitable (Maroli *et al.*, 2013). Several studies from Italy, France, Germany, and the Balkans have demonstrated the emergence of new vector foci and autochthonous *Leishmania* infections (Dujardin J.-C. *et al.*, 2008; Maroli *et al.,* 2013). These trends suggest that northern and eastern European regions may increasingly provide ecologically suitable habitats for sand flies.

In Romania, knowledge of sand fly diversity and distribution remains limited and fragmented (Cazan C.D. *et al*., 2019; Cazan C.D. *et al*., 2021). Although historically considered non-endemic for

[1]Faculty of Veterinary Medicine, Department of Clinics, "Ion Ionescu de la Brad" Iasi University of Life Sciences, Romania

leishmaniosis, Romania has reported sporadic autochtonous human and canine cases, imported human and canine cases, and serological evidence of *Leishmania* exposure in several regions, especially in the southern part of the country (Dumitrache M.O. *et al.*, 2016; Cîmpan A.A. *et al.,* 2019; Birlutiu V. *et al*.,2025; Mihu A. G. *et al*., 2025).

To date, at least nine sand fly species have been recorded in Romania, including known vectors of *Leishmania infantum* such as *Phlebotomus perfiliewi*, *Ph. neglectus*, and *Ph. balcanicus* (Nitzulescu V., 1929; Cazan C.D. *et al.,* 2019; Andronic B.L. *et al.,*2024). However, because there is no systematic surveillance and only a few recent field studies, it is difficult to accurately assess the country's vector ecology and the related epidemiological risks.

Effective surveillance of phlebotomine populations requires standardized, efficient and comparable trapping methodologies. Classical reviews and modern evaluations emphasize that trap performance varies significantly depending on environmental conditions, species-specific behavior, and trap design. Sticky traps, though historically useful for presence/absence assessments, often exhibit low quantitative efficiency and high dependence on microclimatic variability. On the other hand, CDC light traps remain widely used in Europe due to their established reliability and ability to generate continuous, comparable datasets. More recently, LED-based traps have gained the attention for their operational advantages, including reduced energy consumption, enhanced portability, and lower non-arget catches (Alexander B., 2001; Signorini M. *et al.,*2013; Alten B. *et al.,* 2015; Gaglio G. *et al.,*2017; Gaglio G. *et al.,* 2018; Elnaiem D.-E. *et al.,* 2020; Zorilla V.O. *et al.,* 2021; Somwang P. *et al.,* 2021).

Giving the emerging epidemiological relevane of leishmaniosis in Romania, and the scarcity of the entomological data, updates on sand fly distribution and trap performance are essential. The present study aims to address these gaps by comparatively evaluating the efficiency of three trapping methods - the New Standard Miniature CDC Light Trap (John W. Hock Company, USA), Laika Trap 4.0 (LaikaLab, Pozzuolo, Italy) and sticky traps (made in-house) – across multiple sites in northeastern and southeastern Romania over a three-year period (2023-2025). By combining local field results with existing European knowledge on sand fly ecology and sampling methods, this study offers essential evidence for improving Romania's national surveillance capacity and guiding future assessments of vector-borne disease risks.

MATERIAL AND METHOD

Study area and sampling period

Field sampling was conducted from May to September during 2023, 2024, and 2025, in13 sites across northeastern Romania (Suceava, Botoșani, Iași County) and southeastern Romania (Brăila, Tulcea, Constanța County) (figure 1).

These sites included peri-urban environments, agricultural landscapes, and rural areas near animal shelters - locations known to provide suitable microhabitats for sand fly resting and breeding.

Sampling nights were chosen during periods when the mean nighttime temperature exceeded 15°C, reflecting optimal conditions for sand fly activity.

All traps were placed in shaded, wind-protected areas rich in organic matter, with crevices, or nearby animal shelters - conditions favorable for sand fly activity. Each site was sampled for at least two consecutive nights, with traps operating from dusk to dawn.

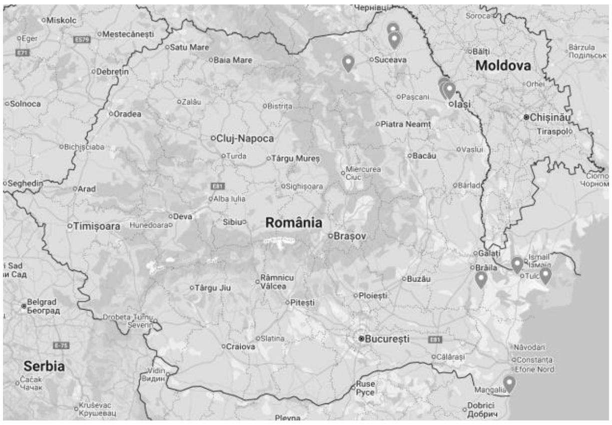

Figure 1. **Sampling sites in Northeastern and Southeastern Romania during the 2023–2025 field campaigns**

Trapping methods

The New Standard Miniature Incandescent CDC Light Trap - Model 1012 (John W.Hock Company, USA) uses an incandescent bulb to attract sand flies, combined with a downdraft suction mechanism driven by a fan. It is equipped with a programmable photoswitch, allowing automatic operation between dusk and dawn. Due to its robustness and wide use in entomological surveys across Europe, it serves as a reference method.

Laika Trap 4.0 (LaikaLab, Pozzuolo, Italy) employs LED-based illumination (white and UV wavelengths) alongside a low-noise fan system. Its compact design and low energy consumption make it ideal for remote field sites. The trap includes a fabric collection bag for easy retrieval and can operate on both 220 V and external battery power.

Sticky traps consisted of A4 sheets coated with castor oil–based adhesive. Between 10 and 20 sheets were deployed per site. Although widely used in presence–absence studies, these traps are known to be sensitive to environmental factors such as humidity and wind.

A comparative overview of the main characteristics of the three trapping methods used in this study is presented in Table 1.

Table 1

Comparative characteristics of the three trapping methods used in this study.

Feature	CDC Miniature Light Trap (Model 1012)	Laika Trap 4.0	Sticky Traps
Principle	Incandescent light + fan	LED light + fan	Passive adhesive
Energy source	220 V or 12 V DC battery	220 V or 6 V DC battery	None
Energy consumption	~0.5 A / h	< 0.3 A / h	0 A / h
Efficiency	Very high (reference method)	High (similar to CDC)	Low
Selectivity	Captures many non-target insects	More selective, fewer non-targets	Low selectivity
Noise level	Moderate	Very low	Silent
Portability	Medium	High	Very high
Cost	Medium	Medium	Very low
Advantages	High efficiency, standardized, robust	Selective, energy-efficient, lightweight	Cheap, simple, no power required
Limitations	Heavier, requires higher power input	Requires 6 V battery for field use	Low catch rate; affected by wind and humidity
Recommended use	Routine entomological surveillance	Remote sites and long-term monitoring	Presence detection only

Specimen Processing and Identification

Captured sand flies were preserved in 70% ethanol. Species identification was performed morphologically under a Zeiss Stemi 305 stereomicroscope using established taxonomic keys for phlebotomine sand flies (Lewis D.J., 1971; Dantas-Torres F., *et al.,*2014).

RESULTS AND DISCUSSIONS

A total of 55 specimens of adult sand flies were collected from 13 monitored sites located in northeastern and southeastern Romania in the summers of 2023, 2024 and 2025. The three trapping methods used in this study showed notable differences in performance. The Laika Trap 4.0 yielded the highest number of captures, collecting 28 specimens (approximately 51%), followed closely by the CDC Light Trap, which captured 25 specimens (approximately 45%). In contrast, the sticky traps captured only 2 specimens (approximately 4%). Thus, more than 95% of all sand flies collected were captured by the two light-based traps, clearly demonstrating their superior efficiency comopared with the passive adhesive method.

Field observations also revealed pronounced operational differences between the traps. The CDC Miniature Light Trap, a standard reference in entomological surveys, performed reliably under all field conditions, offering stable airflow, strong attraction through incandescent illumination, and reproducible operation, although with higher energy use. The Laika Trap 4.0, despite delivering comparable efficiency, showed several practical advantages, including very low noise, reduced energy consumption due to its LED-based dual-wavelength system, and enhanced portability. Its compact design and ability to operate both on mains power and on a 6 V external battery, together with the possibility of connecting up to ten traps in series, made it particularly suitable for extended monitoring in remote areas. Sticky traps, although inexpensive and easy to deploy, were strongly affected by environmental conditions such as wind and humidity, which likely contributed to their poor capture performance. Their passive functioning rendered them inadequate for quantitative surveillance, although they may still be useful for rapid, low-cost detection of sand fly presence in areas without access to electricity.

Overall, the performance patterns observed in this study align with the results of other studies, in which light-based traps consistently outperform adhesive trapping methods, especially in regions situated near the ecological limits of sand fly distribution (Signorini M. *et al.,*2013; Alten B. *et al.,* 2015; Somwang P. *et al.,*2021). Such concordance strengthens the conclusion that light-based active trapping should form the basis of sand fly surveillance in Romania. Given their documented efficiency, technical reliability, and adaptability to different field conditions, both the CDC and Laika traps represent suitable tools for routine vector monitoring, while sticky traps may serve only as complementary devices in specific settings.

CONCLUSIONS

The results of this study show that light-based active traps remain the most efficient methods for collecting phlebotomine sand flies in Romania. Both the CDC Miniature Light Trap and the Laika Trap 4.0 performed similarly and captured almost all specimens, while sticky traps had a very low capture success and are not suitable for quantitative surveillance. The practical benefits of the Laika Trap 4.0, such as lower energy use, reduced noise, and better portability, make it a useful modern alternative for field monitoring, especially in areas with limited access to power.

The findings are consistent with observations reported in other field studies worldwide, supporting the idea that light-based traps should be the main method used for vector monitoring programs. Regular and standardized vector surveillance protocol is important for detecting sand fly presence and for evaluating the potential risk of leishmaniosis, especially in a changing environment. Strengthening these monitoring efforts will help identify shifts in vector distribution at an early stage and support a One Health approach, where human, animal, and environmental health are closely connected.

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