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# Species Composition and Blood Meal Preference of Sandfly (Diptera: Psychodidae) Populations in Bobo-Dioulasso and Larama, Two *Leishmania* Circulating Areas in Western Burkina Faso

**Achille Sindimbasba Nikièma[1*], Diakourga Arthur Djibougou[1,2*], Lassane Koala[1], Ali Ouari[2], Jean-Jacques Tioyé[1], Aristide Sawdetuo Hien[1], Ibrahim Sangaré[3], Florence Fournet[4], Roch Kounbobr Dabiré[1#]**

[1]Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Bobo-Dioulasso, Burkina Faso
[2]Centre Muraz, Institut National de Santé Publique, Bobo-Dioulasso, Burkina Faso
[3]Institut Supérieur des Sciences de la Santé, Université Nazi Boni, Bobo-Dioulasso, Burkina Faso
[4]UMR-MIVEGEC-IRD, Montpellier, France
Email: [#]dabireroch@gmail.com

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## Abstract

The aim of this study was to examine sandfly population patterns in Bobo-Dioulasso and Larama, Burkina Faso, where canine and cutaneous leishmaniasis still occur. We characterized the species composition, blood meal origin, and infection status of a sample of sandflies obtained from cross-sectional surveys. Adult sandflies were collected using CDC light traps placed indoors and outdoors of human dwellings and also using morning pyrethrum spray collections. A subsample from each setting was morphologically identified using the keys of Abonnenc. The *Leishmania* infection status of sandfly females was assessed by Polymerase Chain Reaction, and the blood meal sources of blood-fed females were characterized using ELISA. A total of 976 sandflies were collected at the two sites, predominantly from Larama (944 vs. 32 in Bobo-Dioulasso). Three species of *Phlebotomus* were identified: *Ph. duboscqi, Ph. bergeroti, and Ph. rodhaini*. Eighteen species of *Sergentomyia* were identified, with *Se. schwetzi* being the most frequent (43.75%). Females of both genera fed on a mix of human and animal blood. None of the female sandflies tested were infected with *Leishmania* at either site. This study reports the presence

[*]These authors contributed equally to this work.
[#]Corresponding author.

of *Ph. rodhaini* in Burkina Faso for the first time. Although no infected sandflies were detected, it is still possible that infected females or existing reservoirs could maintain disease transmission. Therefore, an integrated approach using multiple strategies, including a One Health approach, is needed to better survey and control sandfly-borne diseases.

**Keywords**

Sandflies, Frequency, Blood Meal Origin, Infectivity Status, Burkina Faso

## 1. Introduction

Sandflies are small hematophagous Diptera, belonging to the *Phlebotominae* subfamily, which comprises about 800 species described to date [1]. They are vectors of several pathogens, such as *Leishmania* parasites, bacteria of the genus *Bartonella*, and arboviruses [2] [3]. Leishmaniasis are neglected tropical diseases caused by a group of parasites belonging to the *Leishmania* genus. The parasites are transmitted to human hosts by infected female sandflies [4]. Leishmaniasis is responsible for 1.8 million disability-adjusted life years and 26,000 - 65,000 deaths annually and is currently considered the third most important vector-borne parasitic disease after malaria and lymphatic filariasis, causing high mortality [5]-[7]. Clinically, there are three main forms of leishmaniasis: cutaneous leishmaniasis, visceral leishmaniasis, and mucocutaneous leishmaniasis. Visceral leishmaniasis, also known as kala-azar, is the most severe form. Most cases of visceral leishmaniasis occur in Brazil, East Africa, and India. Annually, the World Health Organization (WHO) estimates that 50,000 to 90,000 new cases occur worldwide [7].

In Burkina Faso, the human cutaneous leishmaniasis form, locally called "Maladie de Ouaga 2000," has been the most common since the country's independence in 1960 [8]. *Leishmania major* was the first species molecularly identified, with MON-26 and MON-74 being the main zymodemes involved in cutaneous leishmaniasis transmission [9] [10]. The first location of Human Cutaneous Leishmaniasis (HCL) was Ouagadougou, situated in the center of the country. The rate of parasitological confirmation varied between 2% and 92.5% [11]. After the first outbreak in Ouagadougou, a second focus of HCL was reported in the village of Larama, located 40 km from Bobo-Dioulasso in the western part of Burkina Faso. In 2013, over 580 cases of HCL per 10,000 people occurred in the rural village of Larama, with *L. major* being responsible for the cutaneous leishmaniasis infection [12]. In addition to the human cutaneous foci, a new focus of canine leishmaniasis (CnL) was identified in Bobo-Dioulasso, with two species, *L. infantum* and *L. major*, being implicated [13] [14].

If epidemiological surveys presented an extensive overview of leishmaniasis endemicity in the country, only a few entomological studies were available to support the epidemiology and disease transmission patterns [12] [15]. Recent ento-

mological data on leishmaniasis vectors are lacking, and existing data mainly focus on the Ouagadougou areas [16]-[18]. The first entomological surveys were conducted in 1970 and reported 23 species of sandflies, including *Phlebotomus duboscqi* and *Ph. bergeroti*, as the probable vectors of *L. major* in Burkina Faso (formerly Haute Volta) [16]. In 2005, an entomological survey reported the presence of *Ph. longicuspis*, a proficient vector of *L. infantum* in Ouagadougou [18], along with *Ph. duboscqi* as a vector of *L. major*. Previous investigations had not detected any infected sandflies in the surveyed areas [17]. Following the HLC outbreak in Ouagadougou, HLC remained relatively low nationally until 2013, when a localized outbreak was reported in Larama, a rural village in the Bobo-Dioulasso health region [12]. Unfortunately, no entomological investigation was conducted to complement serological and parasitological data related to the HCL cases in Larama and the CnL cases in Bobo-Dioulasso. Recently, the WHO recommended research to better understand leishmaniasis vector behavior, which can aid in supporting vector control strategies [19]. This highlights the need for entomological studies in leishmaniasis foci to characterize sandfly populations and assess their role in disease transmission.

The study was designed to provide an overview of the abundance, species composition, blood meal preference, and infection status of sandfly populations collected in the urban city of Bobo-Dioulasso and the rural area of Larama, both endemic to CnL and HCL respectively in Burkina Faso.

## 2. Materials and Methods

Study sites

Entomological surveys were conducted during the rainy season from July to September 2018 in the village of Larama (34.72˚N, −5.65˚W) within the health district of Soumousso, located 25 km from Bobo-Dioulasso. The area features wooded savannah interspersed with gallery forests [12]. The majority of Larama's population (1,853 inhabitants) are farmers and/or pastoralists. The annual rainfall varies between 1,000 and 1,300 mm, and the average annual temperature is 28˚C.

The Bobo-Dioulasso district selected for surveys is an urban area (34.72˚N, −5.65˚W) where CnL have been reported recently without any information about their potential vectors [13] [14]. **Figure 1** shows the location of the study sites.

Sampling of sandfly populations

In each site, two methods were used to collect adult sandflies both indoors and outdoors, as described previously [20].

CDC light traps were placed indoors and outdoors near houses (bovine and poultry houses) for five consecutive nights each month during the study period. The traps were installed before sunset and retrieved the following morning (6:00 p.m. to 9:00 a.m.) to align with the activity periods of sandflies: dusk and dawn. A total of 16 traps were used, with eight indoors and eight outdoors. These locations were primarily houses where positive cases in humans or dogs had been reported. The traps were then removed and taken to the Entomology Laboratory of IRSS/Centre MURAZ to sort the collected sandflies.

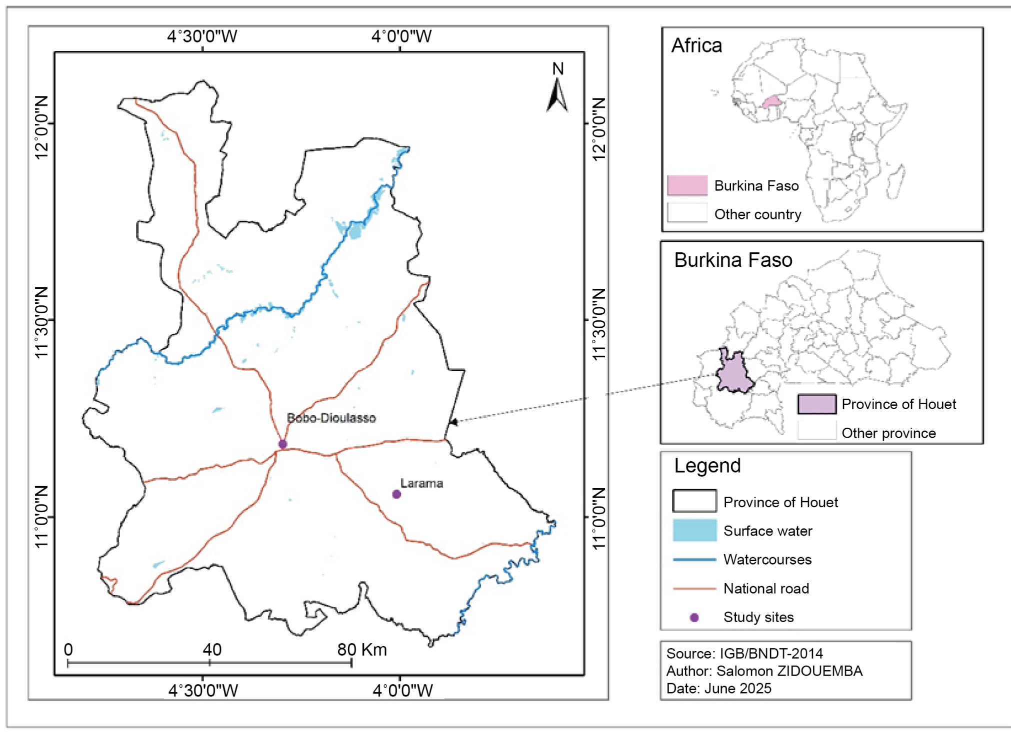


**Figure 1.** Study sites location in province of Houet, Burkina Faso (source: IGB/BNDT-2014).

Pyrethrum Spraying Collection (PSC) was conducted by spraying aerosol indoors in selected rooms for five consecutive days at each site between 6 am and 9 am. After spraying, rooms were kept closed for 7 - 12 minutes. Sandflies were collected, placed in labeled Petri dishes containing blotting paper soaked in water, and transported to the Entomology Laboratory.

Identification of the sandfly species

All sandflies collected were sorted for species identification. Initially, we planned to identify the species using RT-PCR, but we couldn't establish this method in our molecular laboratory. Therefore, all females were used for DNA extraction for RT-PCR to determine species identification and infection status. However, since the species identification protocol was not successfully implemented, species identification was performed morphologically using male specimens, based on differences in genitalia, bristles, and spicules. Males were randomly selected and transferred individually into 1.5 mL tubes containing 96% ethanol and labeled for species identification. The remaining males were also transferred individually into 1.5 mL tubes containing 96% ethanol, labeled, and stored at −80˚C. Females were morphologically identified to the genus level and then stored to determine the origin of their blood meal sources and their infection status with *Leishmania* species.

For the taxonomic identification of the species, we used the determination key of Abonnenc [21] and a CD-ROM titled "*Les phlébotomes d'Afrique de l'Ouest: logiciel d'identification et d'enseignement*" from the *Institut de Recherche pour le Développement* (IRD), Edition 2004 [22]. For each specimen, the head and geni-

talia were mounted in Euparal after undergoing several baths: 4 hours in 10% potassium hydroxide and fuchsin, 2 hours in distilled water, 10 hours in a Marc-André solution, 10 hours in distilled water, 20 minutes in 70% ethanol, 20 minutes in 90% ethanol, 20 minutes in 96% ethanol, and 10 hours in a clove oil solution.

Determination of sandfly females' blood meal origin

The abdomens of 31 fed females were cut, crushed into a homogenate, and subjected to ELISA analysis to determine the origin of their blood meal according to the method of Beier *et al.* (1988) [23]. The antibodies tested were those of cattle/sheep/goat, human, and pig.

Molecular screening for *Leishmania* spp infection in sandflies by RT-PCR.

DNA was extracted from the head and thorax of each female sandfly using 2% cetyl trimethyl ammonium bromide (2% CTAB). The DNA from each specimen was then pooled based on the females' repletion status (fed, unfed, gravid), capture method, capture site, and with an average of 19 sandflies per pool, ranging from 1 to 128 females, as described by Anderson *et al.* [24]. A volume of 2 µL of DNA from each sample was used to create 24 pools (P): P1 to P24.

The STAT-NAT *Leishmania* spp kit (Sentinel Diagnostic, Milano-ITALY), a lyophilized RT-PCR mixture, was used to amplify the kinetoplast DNA (kDNA) minicircles to detect *Leishmania* spp. The amplification was performed on the ABI 7500 RT-PCR system with 1 cycle at 95˚C for 10 minutes, followed by 45 cycles at 95˚C for 15 seconds and 45 cycles at 60˚C for 60 seconds. The *Leishmania* spp (FAM) and the internal control (IC) (JOE) probes were analyzed individually at the end of the amplification. The cycle threshold (CT) of the amplified fragments was normalized using the Internal Control (IC) and ranged from 15 - 38 for positive results, whereas a normalized CT > 38 was considered negative [14].

Data analyses

Data were entered into an input mask using Sphinx software version 5, and statistical analysis was conducted using Stata 12.1 software. The proportion of each genus was estimated by dividing the number of a given sandfly by the total count of collected genus, then multiplying by 100 [25].

## 3. Results

Characteristic of the sandfly fauna of Larama and Bobo-Dioulasso

A total of 976 (279 males and 697 females) were collected from both sites: 944 (96.7%) from Larama and 32 (3.3%) from Bobo-Dioulasso. Two genera of sandflies were collected, with the *Sergentomyia* genus accounting for 94.4% (921/976) and the *Phlebotomus* genus for 5.6% (55/976).

Sandfly species composition

A total of 189 male (169 *Sergentomyia* and 20 *Phlebotomus*) were randomly selected to be identified at the species level from all the samples collected at the two sites.

The identification reported the presence of 18 species belonging to the genus *Sergentomyia* and three species belonging to the genus *Phlebotomus*. Among the

*Sergentomyia* species, *Se. schwetzi* was the most abundant, comprising 43.8% (n = 92), and was observed with endophilic behavior (collected indoors) in Bobo-Dioulasso as well as in Larama. This species was followed by *Se. buxtoni* (12.5%, n = 24), *Se. ghesquieri* (5.7%, n = 11), and *Se. distincta* (4.2%, n = 8). Other species of the *Sergentomyia* genus were found in very low proportions, comprising less than 20% overall: *Sergentomyia africana* (0.5%, n = 1), *Se. squamipleuris* (1.6%, n = 3), *Se. clydei* (2.6%, n = 5), *Se. ingrami* (1.0%, n = 2), *Se. adleri* (1.0%, n = 2), *Se. adami* (1.0%, n = 2), *Se. antennata* (1.0%, n = 2), *Se. diapgai* (2.1%, n = 4), *Se. magna* (1.0%, n = 2), *Se. dureni* (1.6%, n = 3), *Se. dissimillima* (2.6%, n = 5), *Se. wansoni* (0.5%, n = 1), and *Se. christophersi* (1.0%, n = 2). Belonging to the genus *Phlebotomus*, only three species have been identified: *Phlebotomus (Ph.) duboscqi*, which was the most represented (9.3%, n = 18), followed by *Ph. bergeroti* and *Ph. rodhaini* in similar proportions (0.5%, n = 1). For the first time, *Ph. rodhaini* has been identified in Burkina Faso from the Larama village outdoor collections.

Blood feeding preference and infection status of female.

A total of 31 fed female sandflies from both sites (25 blood-fed females of *Sergentomyia* and 6 blood-fed females of *Phlebotomus*) were screened for blood meal origin by ELISA according to the method of Beier *et al.* (1988) [23]. Of the 25 blood-fed females of *Sergentomyia*, 12 fed on humans, 12 on other animals, and only one presented mixed human-animal blood meals. The 6 blood-fed females of the *Phlebotomus* genus included 2 human blood meals and 4 animal blood meals (cattle, sheep/goat, pig).

Out of 438 females from both sites tested by PCR for infection status, none tested positive for *Leishmania* spp (Table 1).

Table 1. Infection status of female sand flies pooled according to their repletion status and site origin.

| PCR Pool | Site of capture | Number of sand flies per pool | Repletion status | Results of RT-PCR | Infection rate |
|---|---|---|---|---|---|
| **P1** | Larama_CDC_Out | 9 | *Sergentomyia* bloodfed | N | **0%** |
| **P2** | Bobo_CDC_Out | 2 | *Sergentomyia* bloodfed | N | **0%** |
| **P3** | Larama_CDC_In | 2 | *Sergentomyia* bloodfed | N | **0%** |
| **P4** | Larama_PSC | 18 | *Sergentomyia* bloodfed | N | **0%** |
| **P5** | Bobo_CDC_Out | 1 | *Phlebotomus* bloodfed | N | **0%** |
| **P6** | Larama_CDC_In | 3 | *Phlebotomus* bloodfed | N | **0%** |
| **P7** | Bobo_CDC_In | 1 | *Phlebotomus* bloodfed | N | **0%** |
| **P8** | Larama_PSC | 8 | *Phlebotomus* bloodfed | N | **0%** |
| **P9** | Bobo_PSC | 2 | *Phlebotomus* bloodfed | N | **0%** |
| **P10** | Larama_CDC_Out | 14 | *Sergentomyia* gravid | N | **0%** |
| **P11** | Bobo_CDC_Out | 5 | *Sergentomyia* gravid | N | **0%** |
| **P12** | Larama_CDC_In | 12 | *Sergentomyia* gravid | N | **0%** |

Continued

| | | | | | |
|---|---|---|---|---|---|
| **P13** | Larama_PSC | 12 | *Sergentomyia* gravid | N | **0%** |
| **P14** | Larama_CDC_Out | 5 | *Sergentomyia* gravid | N | **0%** |
| **P15** | Bobo_CDC_Out | 2 | *Phlebotomus* gravid | N | **0%** |
| **P16** | Larama_CDC_In | 2 | *Phlebotomus* gravid | N | **0%** |
| **P17** | Larama_PSC | 2 | *Phlebotomus* gravid | N | **0%** |
| **P18** | Larama_CDC_Out | 128 | *Sergentomyia* unfed | N | **0%** |
| **P19** | Bobo_CDC_Out | 2 | *Sergentomyia* unfed | N | **0%** |
| **P20** | Larama_CDC_In | 181 | *Sergentomyia* unfed | N | **0%** |
| **P21** | Larama_PSC | 13 | *Sergentomyia* unfed | N | **0%** |
| **P22** | Larama CDC Out | 1 | *Phlebotomus* unfed | N | **0%** |
| **P23** | Larama CDC_In | 3 | *Phlebotomus* unfed | N | **0%** |
| **P24** | Larama_PSC | 10 | *Phlebotomus* unfed | N | **0%** |
| **Total** | | **438** | | **N** | **0%** |

P1 = Pool1, S. = Sergentomyia; P. = Phlebotomus, Out = Outdoor, In = Indoor, PSC= Pyrethrum Spraying Collection, N = negative.

## 4. Discussion

Overall, sandflies were unexpectedly abundant in our collections, with high numbers (n = 976) observed in both study areas. We did not identify all 976 specimens due to limited laboratory expertise and equipment for analyzing large quantities. Therefore, we randomly sampled only 200 male individuals, which seemed easier to identify based on identification keys, while females were kept to determine infection status by PCR. Of the 200 male specimens, 196 were successfully dissected, treated, and analyzed for morphological criteria.

The results showed that the genus *Sergentomyia* was the most abundant (94.4%) and diverse in terms of the number of species identified at both sites, followed by the genus *Phlebotomus* (5.6%). *Sergentomyia* appeared to be the predominant genus, as it was mostly sampled in Ouagadougou in previous surveys in Burkina Faso [17] [18] [26]. It is also widespread in West Africa, as revealed by studies in Senegal (93.7%), Mali (98.1%), Côte d'Ivoire (91%), and Chad (99.4%) [25] [27] [28]. With the extensive use of insecticide-treated nets (ITNs) for malaria vector control and pesticides for crop protection, one might anticipate a decline in sandfly populations, assuming they are vulnerable to the insecticides used for public health and crop protection [29]. The worst issue may be the emergence of insecticide resistance within sandfly populations, which could lead to their proliferation and increase the risk of disease transmission. Malaria vector control interventions could be the best way to control sandfly populations as part of integrated vector interventions [30] targeting malaria, lymphatic filariasis, and leishmaniasis vectors [22] [31].

At the species level, *Se. schwetzi* was the most abundant species found in our samples, confirming its wide distribution in West Africa and the Afrotropical re-

gion [17] [18] [20] [24], supported by its ability to feed on both humans and animals [20] [32].

*Ph. duboscqi,* well established as a vector of *Leishmania major* in West Africa [11] and very common in Burkina Faso, was found in our study sites. Additionally, even in low proportions, *Ph. bergeroti* and *Ph. rodhaini* were also found, particularly in Larama, highlighting the diversity of sandfly species in Burkina Faso. Notably, this is the first time *Ph. rodhaini* has been reported in Burkina Faso, adding to the list of sandflies [16]. This species is generally considered rare in neighboring countries like Mali and Senegal [20] and may also play a possible role in maintaining the transmission of *Leishmania donovani* between animal hosts in eastern Sudan [33]. There is no evidence in the literature that *L. donovani* is present in Burkina Faso [34]. However, the presence of a competent vector, *Ph. rodhaini*, indicates a potential risk of visceral leishmaniasis in Larama. Raising awareness among healthcare professionals is crucial. The signs of visceral leishmaniasis, such as splenomegaly and fever, are similar to those of malaria, which is endemic in Burkina Faso. Cases of visceral leishmaniasis could therefore be mistaken for and treated as malaria. It is essential to establish and strengthen an effective monitoring system for leishmaniasis within the healthcare system and continue entomological surveillance of leishmaniasis in Larama.

Bloodmeal analysis showed that blood-feeding sandflies, *Sergentomyia* and *Phlebotomus* genera, were anthropophilic, zoophilic, and anthropozoophilic. These findings support the hypothesis that these sandflies, which are anthropophilic or zoo-anthropophilic, are likely responsible for the transmission of *Leishmania* in these two localities. No infected females were found on the head and thorax of the flies. This could be a limitation of the method we used. Perhaps, instead of using the head and thorax, we could have detected *Leishmania* by using the whole sandfly.

Of the 438 female sandflies analyzed by PCR, none showed infection with *Leishmania* spp. This does not necessarily exclude the circulation of *Leishmania major* and *L. infantum* in these areas. The polymerase chain reaction (PCR) has been shown to enhance the sensitivity of the diagnostic process compared to the conventional approach of infection assessment through dissection. The dissection process is laborious and must be conducted in the field on specific tissues like the midgut of the sandfly [35]. In regions with low infection prevalence, meticulous examination of multiple sandflies is necessary to confirm the presence or absence of infection. Even if the staff is well-trained in dissection, it is likely that two technicians may obtain conflicting results [35]. The negative PCR results may be due to low infection rates in humans and/or non-human reservoirs, resulting in low transmission rates undetected by this study. Additionally, the study's collection period might not have aligned with the peak abundance of the vector species. This finding may suggest the existence of other species not captured in our study or that infected vectors are present, but we did not collect infected females. The study focused exclusively on the vector, without simultaneous investigation of the res-

ervoir host. It is hypothesized that conducting parallel parasitological investigations in hosts (both human and non-human reservoirs) and entomological surveys could provide a more comprehensive understanding of the circulation patterns of *Leishmania* parasites.

The potential risk of outbreaks in these sites is supported by the abundance and circulation of zoo-anthropophilic *Sergentomyia* species. In Ghana, Nzelu *et al.* (2014) reported cases of natural infection in three species of the genus *Sergentomyia* (*Se. ingrami*, *Se. africana*, and *Se. hamoni*) [36]. In Senegal, using ecological, parasitological, and molecular studies, Senghor *et al.* (2016) provided evidence for the possible transmission of *L. infantum* to humans and dogs via *Sergentomyia* sandflies (*Se. dubia* and *Se. schwetzi*) [34]. Studies conducted in cutaneous leishmaniasis foci in Iran, Mali, and Portugal have shown the detection of *L. major* DNA in *Se. sintoni* [37], *Se. darlingi* [38] and *Se. minuta* [39], respectively.

The circulation of *Ph. duboscqi*, *Ph. bergeroti*, and *Ph. rodhaini* as competent vectors of *L. major* and *L. donovani*, respectively [11] [27], and concurrently with *Sergentomyia* species, highlighted the presence of the CnL reservoir in Bobo-Dioulasso and the epidemiological chain of HCL in Larama. One limitation of our study could be the short duration of the sampling period, which did not allow for optimal collection of species and infected females. Additionally, the morphological identification of sandfly species focused only on male specimens, reducing the representativeness of minor species or potentially ignoring their presence if no males were in the sample analyzed. Further studies could extend the identification process to include female sandflies. Nevertheless, the current study was important as it allowed us to update the sandfly fauna and tentatively monitor their *Leishmania* infection status.

To better control sandfly populations, an integrated vector control approach is recommended. This could include multiple simultaneous interventions. Transmission is part of a complex ecosystem involving human hosts, animal reservoirs, parasites, and vectors. These strategies should combine (i) vector control targeting sandflies, (ii) xenosurveillance of the disease, (iii) control of animal reservoir hosts, and (iv) social mobilization and strengthening partnerships [40].

Our study identified sandfly species from urban and rural settings in Burkina Faso, even though identification was performed only with male specimens. *Se. swertzi* and *Ph. duboscqi*, well known as competent species involved in the transmission of CnL and HCL, respectively, have been reported in this study. For the first time, *Ph. rodhaini*, a possible vector of visceral leishmaniasis, has been reported in Burkina Faso. Blood-meal analysis indicated that sandflies were anthropophilic as well as zoophilic and could also be anthropo-zoophilic. However, we did not detect *Leishmania* parasites within vectors, which does not exclude the possibility of active circulation of infected females within vector populations. Developing innovative strategies for the surveillance and monitoring of leishmaniasis and malaria, and formulating an integrated vector control strategy, are required.

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## Author Contributions

Achille Sindimbasba Nikièma (Methodology [Equal], Formal Analysis [Equal], Writing-original draft [Equal], Writing-review and editing [Equal]); Diakourga Arthur Djibougou (Methodology [Equal], Software [lead], Validation [Equal], Formal Analysis [Equal], Investigation [Equal], Data curation: [Equal], Writing-original draft [Equal], Visualization [Equal], Writing-review and editing [Equal]); Lassane Koala (Methodology [Equal], Writing-review and editing [Equal]); Ali Ouari ( Methodology [Equal], Investigation [Equal], Writing-review and editing [Equal]); Jean Jacques Tioyé (Methodology [Equal], Investigation [Equal], Writing-review and editing [Equal]); Aristide Sawdetuo Hien (Methodology [Equal], Writing-review and editing [Equal]); Ibrahim Sangaré (Visualization [Equal], Writing-review and editing [Equal]); Florence Fournet (Visualization [Equal], Writing-review and editing [Equal]); Roch Kounbobr Dabiré (Conceptualization [Lead], Methodology [Lead], Validation [Lead], Formal Analysis [Lead], Validation: [Lead], Resources [Lead], Visualization [Lead], Project administration [Lead], Writing-original draft [Lead], Writing-review and editing [Lead]).

## Ethic approval

The survey did not need any formal ethic approval. Aerosol insecticide spraying indoors was carried out with the permission and cooperation of the household owners.

## Availability of Data and Materials

The dataset generated and analyzed during the current study are available from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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## Abbreviations

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|---|---|
| DNA | Deoxyribonucleic Acid |
| CnL | Canine Leishmaniasis |
| ELISA | Enzyme-Linked ImmunoSorbent Assay |
| HCL | Human Cutaneous Leishmaniasis |
| IC | Internal Control |
| IRD | *Institut de Recherche pour le Développement* |
| WHO | World Health Organization |
| *Ph* | *Phlebotomus* |
| *Se* | *Sergentomyia.* |