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Research Article

Occurrence and monthly dynamics of female Phlebotomine sandfly species from Kousseri Visceral Leishmaniasis endemic focus in northern Cameroon

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Abstract

Leishmaniasis, an anthropozoonotic infection transmitted to humans and animals through female sandfly bite, is endemic in the far north region of Cameroon, which currently lacks a control programme. This study aimed to assess the occurrence and monthly distribution of sandflies in Kousseri County, a region with reported cases of visceral leishmaniasis (VL), to provide data needed for combatting the disease. Sandfly occurrence was evaluated by monthly deployment of six CDC light traps over a twelve-month period (from September 2020 to August 2021) in urban, peri-urban and rural ecotopes. Our survey collected 4,214 sandflies, including 51.02% females. The highest abundance of sandflies was recorded in the rural areas (43.43%, n = 1830) and in January during the dry season (16.22%, n = 679). Ten sand fly species from the *Sergentomyia* genus Franca and Parrot 1920 and one species of the *Phlebotomus* genus Rondani and Berté 1840 were identified. The monthly dynamics of the collected female sandflies varied markedly: *Se. antennata* Newstead, 1912, the most prevalent species and *Se. squamipleuris* Newstead, 1912 showed unimodal peaks in January and February, respectively; *Se. schwetzi* Adler, Theodor & Parrot, 1929 exhibited a trimodal pattern with a major peak in September; *Se. distincta* followed a bimodal trend with peaks in January and May. The aforementioned sandfly species were present throughout the study period, while other species displayed variable and intermittent presence. *Ph. duboscqi*, the sole known vector of leishmaniasis in this study, was absent in January and July, but peaked in September, representing only 1.07% of the total catch. The Shannon–Wiener index was high ($H > 1$) in all the collected ecotopes and months, indicating high evenness and Simpson indices. Overall, the findings suggest that sandflies are active year-round in Kousseri, with elevated abundance during the dry season, particularly in January and March. The presence of animal shelters surrounding houses, vegetation (primarily rice paddies) and building conditions with unplastered, fissured mud walls likely favour a high abundance of sandflies in rural areas in Kousseri. These findings highlight the need of optimising surveillance and intervention strategies, especially in rural settings and during months

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when sandflies are more active. Further studies on climatic and environmental factors affecting sandfly distribution, as well as investigations into their feeding habits and *Leishmania* infection rates, are crucial for enhancing disease control in the region.

Key words: Kousseri, leishmaniasis, monthly distribution, sandfly species

Introduction

Leishmaniases are anthroponotic and zoonotic parasitic diseases caused by flagellated unicellular protozoa belonging to the genus *Leishmania* Ross, 1903 and transmitted to animals and humans through the bite of haematophagous female phlebotomine sandflies (Akhoundi et al. 2016; Depaquit and Léger 2017). Clinically, leishmaniases encompasses four broad spectrums of syndrome, including cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), mucosal or mucocutaneous leishmaniasis (MCL) and post-kala-azar dermal leishmaniasis (PKDL) (WHO 2017, 2020). These infections disproportionally affect the most impoverished people in many tropical and subtropical countries of the Old World (OW) and New World (NW), occurring almost exclusively in developing countries and are still considered as neglected tropical diseases (Ngouateu and Dondji 2022).

Sandflies, the primarily known vector of leishmaniasis, are Nematocera Diptera insects belonging to the family Psychodidae and the subfamily of Phlebotominae (Dvorak et al. 2018). However, they are also proven, or suspected, vectors of many arboviruses, some of which threaten human and animal health (Jancarova et al. 2023). Approximately 1000 species have been reported in both the Tropics and subtropics, with some species extending into temperate regions in both the Northern and Southern Hemispheres (from 50°N to about 45°S) (Lane 1993; Depaquit and Léger 2017; Maroli et al. 2013; Galati et al. 2025). Two genera in the OW and NW are almost exclusively known as vectors of disease agents: *Phlebotomus* Rondani & Berté, 1840 and *Lutzomyia* França, 1924 sensu lato (s.l.) respectively (Munstermann 2019; Cecílio et al. 2022). *Lutzomyia* s.l. is incriminated or suspected to be vectors of *Leishmania* spp. in the NW comprises several genera: *Lutzomyia* sensu stricto (s.s.), *Psychodopygus*, *Nyssomyia*, *Bichromomyia*, *Migonemyia*, *Trichophoromyia* and *Pintomyia* (Brazil et al. 2015).

The transmission dynamics of leishmaniases are influenced by various ecological and environmental factors, including the distribution and abundance of sandfly vectors (Chaniotis et al. 1971; Falcao de Oliveira et al. 2016). Sandflies characteristically display seasonal and biotopic pattern characteristics (Munstermann 2019). For instance, the activity of sandflies in two CL foci in the Fes-Boulemane Region of northern Morocco was marked by the dominance of *Phlebotomus sergenti* Parrot, 1917, with two peaks occurring in June and September (Talbi et al. 2015). In Jajarm County, an area with a high risk of CL, in north Khorasan, Iran, *Phlebotomus papatasi* Scopoli, 1786 and *Sergentomyia sintoni* Pringle, 1953 were active in all months and were collected with maximum number and percentage in September and August, respectively (Jalali et al. 2022). In Jenin District (Palestinian Territories), *Phlebotomus sergenti* Parrot, 1917 and *Phlebotomus syriacus* Adler & Theodor, 1930 showed two peaks, one in July and the other in October (Sawalha et al. 2017). In semi-arid regions of

Brazil, such as the Atlantic Forest conservation unit, sandfly populations exhibit marked seasonal dynamics influenced by climatic factors, with each species responding differently to climatic conditions (Pinheiro et al. 2021). Targeted interventions during peak months could significantly reduce the population and thereby lower the risk of disease transmission.

Kousseri, located in the Far North Region of Cameroon, is a known endemic focus of VL (Kaptue et al. 1992; Dondji et al. 2001). A sero-epidemiological survey conducted in this locality revealed a seroprevalence rate of 4% amongst school children, indicating the endemicity of VL in the region (Dondji et al. 2001). Despite this known prevalence, the real vector of the disease is still unknown and there are no existing sandfly control measures in Kousseri, highlighting the urgent need for targeted interventions to mitigate disease transmission (Tebo-Nzesseu et al. 2024).

The occurrence of sandflies in Cameroon was first documented by Herve in 1936 (Hervé 1937). However, a more comprehensive listing of sandfly species in the country was compiled by Rageau (1951) and later expanded by Rageau and Adam (1953). The most recent update, compiled by Tateng et al. (2025), identifies 33 species and subspecies of sandflies across four genera and six subgenera of the OW. Amongst these, *Ph. duboscqi* Neveu-Lemaire, 1906 is currently recognised as the only confirmed vector of CL in the region (Dondji et al. 2000; Tateng et al. 2019). However, Tateng et al. (2018) findings have identified *Leishmania donovani*, the causative agent of VL in Sub-Saharan Africa foci (Kone et al. 2019; Yemeli et al. 2021). This discovery raises concerns about its potential role in VL transmission.

To date, there are no existing sandfly control measures in Cameroon (Ngouateu and Dondji 2022) as a whole and in Kousseri in particular (Tebo-Nzesseu et al. 2024). Despite the studies on the seasonal distribution of sandflies in the country (Tateng et al. 2023; Tebo-Nzesseu et al. 2024), no study has attempted to assess the monthly distribution of the species, which is crucial for informing targeted interventions, such as insecticide application or the utilisation of bed nets, to diminish the species population and mitigate disease transmission, particularly during the months when sandflies are most prevalent. Furthermore, it may also help prioritise months for monitoring and intervention initiatives, so optimising the efficacy of limited resources in endemic areas. Understanding the seasonal and spatial distribution of sandflies is critical for developing effective vector control strategies and reducing the risk of disease transmission.

The present study is aimed at understanding the occurrence and monthly distribution of the sandfly populations in Kousseri VL endemic focus. This study may be essential for developing effective control strategies.

Materials and methods

Study area

This study was carried out in Kousseri (12°08'009"N, 15°03'236"E, 317 metres above sea level), a confirmed human VL focus in northern Cameroon (Kaptue et al. 1992; Dondji et al. 2000). Kousseri is the capital city of Logone and Chari Division, located in the far north of Cameroon. The town is bordered to the north and east by the Logone River, which serves as the international border with Chad, to

the south by the Logone-Birni Subdivision and to the west by the Makary Subdivision (Fig. 1). Kousseri has a cosmopolitan population of approximately 101,246 inhabitants, with a land area of about 160 km^2 (PCD 2013). The climate is of the semi-arid Sahelian type with a prolonged dry season lasting eight months (from October to May) and a short rainy season lasting four months (from June to September). The temperature fluctuates accordingly to the month, dropping to 11 °C from December to February and rising to 45 °C in the shade from April to May. The annual rainfall ranges from 500 to 600 mm. A clear, shrubby and thorny savannah dominates the area. Acacias (*Nelotica senegalensis*, Sigal and Seyol), Borabus and stunted shrubs are the dominating tree species (PCD 2013). Livelihood activities in Kousseri primarily include animal husbandry, fishing and agriculture. The Kousseri VL focus likely extends into Chad, including the capital city N'Djamena.

Collection of sandfly species

Field-collected sandfly was conducted monthly during a 12-month period (from September 2020 to August 2021). Collections of sandflies were carried in urban (A, E), peri urban (B, C) and rural (D) areas using miniature CDC (Centers for

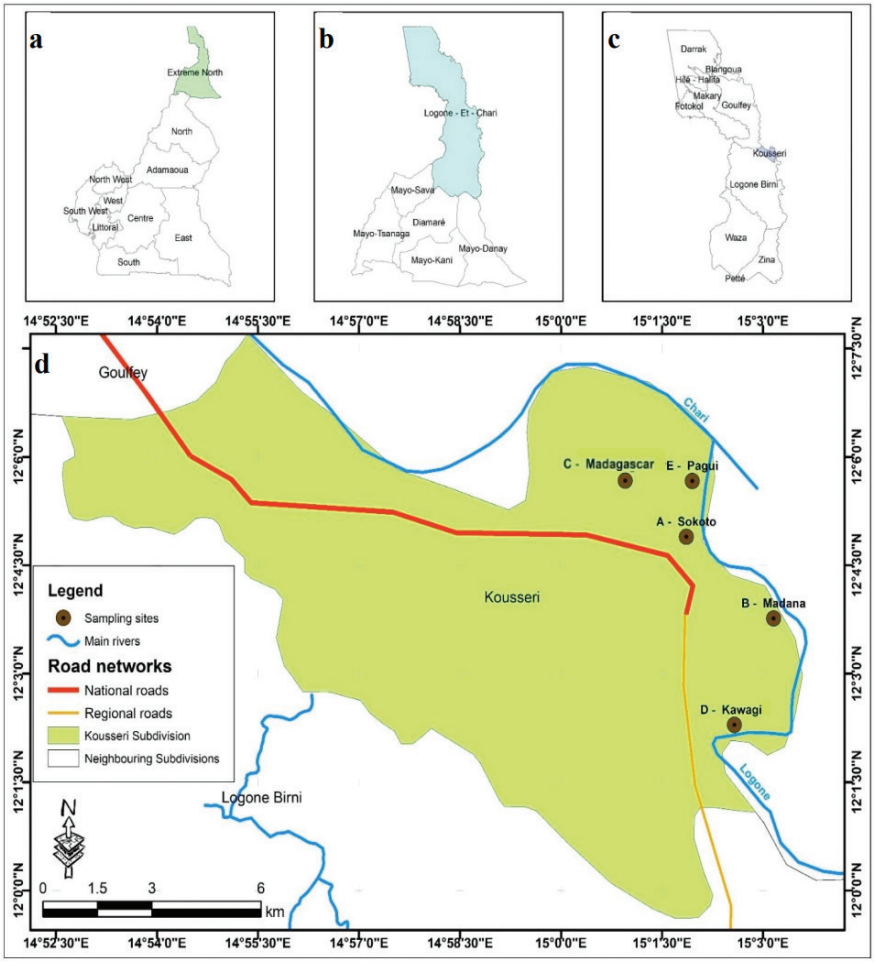

Figure 1. Cameroon map showing the Far North Region (**a**), Logone et Chari Division (**b, c**) with Kousseri (**d**) sandfly sampling sites (**A–E**). **A, E.** Urban; **B, C.** Peri-urban; **D.** Rural (Tebo-Nzesseu et al. 2024). Source: Base map and layers sources: Datums WGS 84, Projection 84 UTM Zone 33 Esri, Sources Cameroon Forest Atlas 2023, Personal data.

Disease Control) light traps equipped with incandescent and black light bulbs. The traps were operational from 6:00 pm to 6:00 am and were strategically placed at heights of 50–80 cm above ground level. A total of six traps were deployed at each study location (three of each trap type) across 30 trapping nights per month.

Study site characteristics

Urban sites were predominantly residential, characterised by dense human occupation and higher levels of urbanisation, featuring a mixture of traditional plastered mud-wall houses and modern infrastructure. In contrast, peri-urban and rural areas exhibited less urbanisation, with traditional mud-wall houses predominating and human dwellings located in closer proximity to domestic animals. Peri-urban areas had denser populations compared to rural areas, which were sparsely settled with larger spaces between compounds. The primary distinction between peri-urban and rural settlements lies in the presence of agricultural activities and vegetation. Rural areas were surrounded by rice paddies, agricultural fields and trees and their inhabitants faced poorer socio-economic and housing conditions compared to peri-urban residents.

The presence of various animal species across the study sites is noteworthy, given their potential role as blood-meal sources for sandflies. Dogs were found in almost all surveyed sites, while cats were observed only in urban areas. In peri-urban regions, livestock such as sheep, goats and chickens were common, with ducks/fowls predominantly present in rural settings. Additional fauna in the area included small reptiles, such as geckos and other lizards, which might also serve as occasional hosts.

Trap Deployment and Site Selection Criteria

Sampling effort was standardised by deploying six CDC traps per environment, ensuring uniform trap density across all sites. This systematic trapping approach provided comprehensive coverage across diverse habitats and allowed for the consistent monitoring of sandfly populations throughout the study period. Capture site selection was conducted following household approval and was guided by the following criteria:

1. The level of urbanisation and socio-economic status of the population.
2. Ecotypes within the region, including the presence of domestic animals, humid and shaded shelters and agricultural fields.
3. Accessibility, security and the availability of lodging facilities.

Sorting of sandflies

Following collection, sandfly specimens were euthanised by freezing at –20 °C for two hours. Once immobilised, they were carefully transferred to sterile Petri dishes for processing. Non-target insects were meticulously removed, and sandflies were segregated into male and female groups under a dissecting microscope. This segregation was primarily based on the examination of the genital armature, a distinctive morphological feature of sand flies (Abonnenc

1972). Each group of sandflies was systematically counted and preserved in labelled cryogenic vials. The preserved specimens were then transported under appropriate storage conditions to the Laboratory of the Leishmaniasis Research Project at the Mokolo Annex Regional Hospital, Mayo Tsanaga Division, in the far north. This laboratory served as the primary facility for the subsequent dissection and accurate taxonomic identification of the collected specimens.

Sandfly dissection and morphological identification

Only caught female specimens were actually dissected by cutting off the head and the posterior half of abdomen with the aids of a microneedle and sterilised forceps and morphologically identified to species level. The dissected organs were immediately slide-mounted in Hoyer's medium (Hem stead Halide TM). After the slides were labelled and air-dried at room temperature for two weeks, species identification was carried out under a stereomicroscope Olympus BX50 at 10–40× using valid diagnostic keys such as described by Abonnenc and Minter (1965) and Abonnenc (1972). To ensure accuracy, identification was cross-validated with more recent updated keys described by Tateng et al. (2019).

Study parameters

The abundance of sandfly specimens was determined by counting all collected individuals per site. Sex ratios were calculated as the number of collected males divided by the number of females (Al-koleeby et al. 2022). Species richness (S), defined as the total number of species collected monthly, was also recorded. Monthly sandfly density, expressed as the number of sandflies per trap per night, was calculated by dividing the total count of sandflies captured by the number of traps deployed per night (Gijón-Robles et al. 2021).

Specific diversity was measured by the Shannon Weiner (H') and the Simpson indices (1-D). The former index measures the probability of encountering a specific species in a stand, expressed as $H' = -\sum_{i=1}^{s} Pi \times \ln Pi$. The Simpson index of diversity measures the dominance of a multispecies community. That is the probability that two individuals randomly selected from a sample belong to the same species. It was calculated as: $(1 - D = 1 - \sum_{i=1}^{s} Pi^2)$. The value of D ranges between 0 and 1, with 0 representing infinite diversity and 1 representing no diversity. That is, the greater the value of D, the greater the sample diversity. To better discuss the Shannon index, it is often accompanied by the Pielou equitability index (J). Pielou evenness expressed as J = H' / log(S), measures the relative abundance of species. J varies between 0 and 1, with higher values indicating greater species evenness. In these formulations, N represents the total number of individuals counted in the sample, S = the total number of species present in the sample; pi = the proportional abundance of a species present (pi = ni/N); ni = the number of individuals in taxon i^{th} (Magurran 1988, 2004).

Statistical analysis

Assumptions of normality and homogeneity of variances were verified prior to analysis, ensuring the robustness of results (Sokal and Rohlf 2012). To ensure comparability, data were standardised using mean flies per trap per night,

minimising biases caused by variable sampling conditions. The abundance of sandfly species was compared across urban, peri-urban and rural ecotopes, with data presented as mean ± standard deviation (SD) to provide an accurate representation of variability and central tendencies. One-way analysis of variance (ANOVA) was performed to identify significant differences in species abundance amongst the three ecological areas.

To analyse seasonal variations, Non-metric Multidimensional Scaling (NMDS), based on Bray-Curtis dissimilarities, was applied to evaluate the similarity in species composition across collection months. Statistical analyses were performed using the MASS package for abundance data (Venables and Ripley 2002) and the VEGAN package for diversity indices (Oksanen et al. 2025) in R software version 4.2.0 (R Core Team 2021).

Results

Sandfly abundance

From September 2020 to August 2021, 4,214 sandfly specimens including 2,150 females (51.02%) and 2,064 males (48.98%) were captured.

Table 1 presents the abundance of the collected sandflies in the capture ecotype. It emerges from this table that the rural area recorded the highest captures of sandflies, accounting for 43.43% (n = 1,830) of the total catches, followed by peri-urban areas, which constituted 33.38% (n = 1,428) of the total captures, while urban environment yielded the least capture represented by 22.69% (n = 956) of the total captures.

Table 2 presents the monthly abundance and sex ratios distribution of sandflies collected in Kousseri. It arises from this table that January and March

Table 1. Abundance of sandflies caught according to the capture area.

	Capture Area			
	Urban (A, E)	Peri-urban (B, C)	Rural (D)	Total (%)
Males	571[a]	694 [a]	799 [b]	2,064 (48.98)[a]
Females	385[b]	734 [a]	1,031[a]	2,150 (51.02)[a]
Total	956	1,428	1,830	4,214 (100.0)
Mean N (%)	22.69	33.88	43.43	100.0

Values affected with different letters in the same column are significantly different ($p < 0.05$), N = abundance, % = percentages, A = Sokoto, B = Madana, C = Madagascar, D = Kawagi, E = Pagui.

Table 2. Monthly abundance and sex ratio distribution of sandflies collected in Kousseri between September 2020 and August 2021.

	Capture months												
	S	O	N	D	J	F	M	A	M	J	J	A	T
Males	72	26	79	188	285	260	365	122	224	105	112	226	2064
Females	75	50	62	124	394	239	232	191	200	206	158	219	2150
Total	147	76	141	312	679	499	597	313	424	311	270	445	4214
SR	1:1.04	1:1.92	1:0.78	1:0.78	1:1.38	1:0.91	1:0.63	1:1.56	1:0.89	1:1.96	1:1.41	1:0.96	1:1.04

SR (male to female sex ratio).

captured the highest number of sandflies (n = 679; n = 597, respectively), while the lowest abundance was recorded in September and October (n = 147; n = 76, respectively. Predominantly female specimens were found in six of the twelve surveyed months including September, October, January, April, June and July with respective SR (sex ratio) of 1:1.04; 1:1.92, 1:1.38, 1:1.56, 1:1.96, 1:1.41. However, males predominate in the other surveyed months (Table 2).

Female Sandfly Species Occurrence and Distribution

From the 2150 female sandflies collected, overall, eleven species belonging to one species from the genus *Phlebotomus* (*Phlebotomus*) *duboscqi* (Neveu-Lemaire, 1906)) and 10 species from the genus *Sergentomyia* (*Sergentomyia antennata* (Newstead, 1912); *Sergentomyia africana* (Newstead, 1912); *Sergentomyia clydei* (Sinton, 1928); *Sergentomyia adleri* (Theodor; 1933); *Sergentomyia distincta* (Theodor, 1933); *Sergentomyia logonensis* (Rageau, 1951); *Sergentomyia affinis vorax* (Parrot, 1948); *Sergentomyia squamipleuris* (Newstead, 1912); and *Sergentomyia inermis* (Theodor, 1938) were morphologically identified (Table 3).

Influence of the ecotype on sandfly species distribution

Table 3 presents the abundance (expressed as mean standard deviation (SD)) and diversity indices of female sandfly species across urban, periurban and rural ecotopes. Looking at this table, excluding *Ph. duboscqi*, which exhibited similar abundances across all ecotopes and *Se. logonensis* and *Se. affinis vorax*, which were present in very negligible numbers, the abundance of other sandfly species displayed notable variations depending on the ecotope.

Table 3. Comparison of species abundance (mean ± SD) and Diversity indices of female sandfly species amongst different ecotopes.

	Ecotopes			
Sandfly species	Urban (A, E)	Peri-urban (B, C)	Rural (D)	Total
Se. antennata	11.42 ± 8.28	23.64 ± 13.7	39.83 ± 55.54	25 ± 34.91
Se. schwetzi	7.08 ± 5.71	26.67 ± 25.39	7 ± 6.37	13.77 ± 17.9
Se. squamipleuris	3.2 ± 2.1	4.2 ± 2.62	18.64 ± 20.59	9 ± 14.06
Se. africana	2.71 ± 1.38	2.6 ± 2.07	8.64 ± 8.98	5.52 ± 6.87
Se. distincta	6.67 ± 4.33	3.18 ± 2.04	1.67 ± 0.82	4.31 ± 3.66
Se. clydei	1.5 ± 0.71	3 ± 1.41	10.11 ± 9.71	7.07 ± 8.34
Se. inermis	3.5 ± 1.22	4.57 ± 3.1	5.5 ± 3.38	4.62 ± 2.82
Se. adleri	1	2 ± 1.73	4 ± 4.38	3.1 ± 3.57
Se. logonensis	4	0	0	4
Se. affinis vorax	0	1	0	1
Ph. duboscqi	1 ± 0	1.44 ± 1.33	1.4 ± 0.89	1.35 ± 1.06
P value	1.29	0.255	0.402	0.722
Diversity indices				
Richness (S)	10	10	9	
Shannon-Weiner (H')	1.68	1.43	1.6	
Simpson (1-D)	0.73	0.62	0.73	
Evenness (J)	0.77	0.68	0.72	

In urban areas, *Se. antennata* and *Se. schwetzi* were the most abundant species. However, *Se. distincta* displayed a particularly higher abundance of 6.67 ± 4.33 of the total catches, compared to only 3.18 ± 2.04 and 1.67 ± 0.82 in peri-urban and rural capture areas, respectively. It is clear that *Se. distincta* abundance greatly decreased from urban to rural areas. This trend suggests a strong preference of this species for urban settings, likely influenced by the availability of human blood meals compared to domestic mammals, which were largely absent in the surveyed sites. However, no significant statistical differences in species abundance were observed in the urban areas (F = 1.29, df = 11, p-value = 0.2). In peri-urban settlements, *Se. schwetzi* exhibited the higher abundance, accounting for 26.67 ± 25.39 contrasting with its reduced number in urban (7.08 ± 5.71) and rural ecotopes (7 ± 6.37). Similarly, *Ph. duboscqi* showed a consistent low abundance of 1.44 ± 1.33 in these settings, suggesting its minimal adaptation to peri-urban environment compared to urban area (1 ± 0), yet no statistically significant differences in sandfly species abundance were observed in peri-urban areas (F = 1.29, df = 11, p-value = 0.2). The number of *Se. antennata*, *Se. clydei*, *Se. adleri*, *Se. africana*, *Se. inermis* and *Se. squamipleuris* significantly decreased from rural to urbanised areas, indicating their adaptation to rural environments. No statistically significant differences in abundance were also noted in sandfly species abundance for rural areas (F = 0.44, df = 11, p-value = 0.92).

Species richness was highest in urban and peri-urban areas (S = 10 species), but slightly lower in the rural area (S = 9 species). Diversity indices, including the Shannon-Wiener index and the Simpson index were highest in urban areas (H' = 1.68; 1-D = 0.77), indicating a more diverse and evenly distributed species composition. The lowest values were recorded in peri-urban areas (H' = 1.43 and 1-D = 0.68), where *Se. schwetzi* and *Se. antennata* dominated.

Influence of months on sandfly species distribution

The monthly distribution of the collected sandfly species from Kousseri varied greatly during the study period (Fig. 2). Species such as *Se. antennata*, *Se. squamipleuris*, *Se. distincta* and *Se. schwetzi* were consistently present throughout all surveyed months, indicating stable activity patterns. Conversely, other species, including *Se. africana*, *Se. inermis*, *Se. adleri*, *Se. clydei* and *Ph. duboscqi*, exhibited periods of absence, suggesting seasonal dormancy or reduced activity.

Amongst the consistently present species, *Se. antennata* demonstrated a unimodal distribution pattern, with its peak activity recorded in January during the dry season, averaging 16.67 sandflies per trap per night. Similarly, *Se. squamipleuris* displayed a unimodal trend, achieving maximum abundance in February, with a mean capture rate of 7.38 sandflies per trap per night. *Se. distincta* followed a bimodal distribution pattern, peaking in January and May, with average capture rates of 2.75 and 2.22 sand flies per trap per night, respectively. However, *Se. schwetzi* exhibited a trimodal distribution pattern, with peaks observed in September (5.25 sand flies/trap/night), March (6.14 sand flies/trap/night) and August (7.62 sand flies/trap/night).

Other species presented diverse abundance trends with absence in some months. Amongst these, *Se. clydei* showed bimodal peaks in February and June, with mean capture rates of 4 and 3.67 sandflies per trap per night, respec-

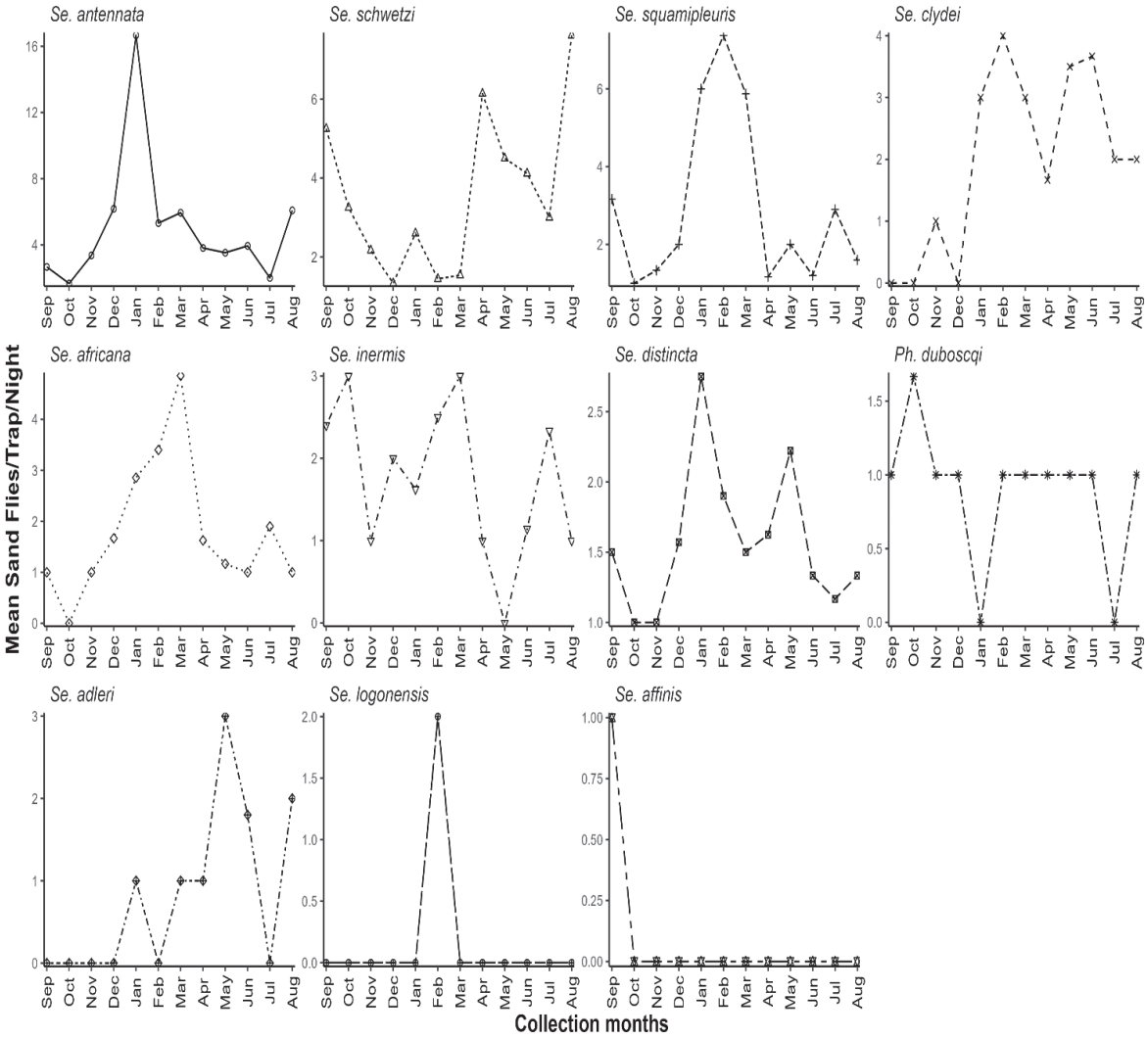

Figure 2. Monthly distribution of female sandfly species collected in Kousseri between September 2020 and August 2021. The x-axis represents the collection months, while the y-axis shows the mean number of sandflies captured per light trap per night.

tively. *Se. africana* reached a unimodal peak during the dry season in March, averaging 4.86 sandflies per trap per night.

Ph. duboscqi, the sole representative of the genus *Phlebotomus* in this study, despite its very low overall abundance (1.07%, n = 23), displayed a unimodal distribution trend with a peak in September, accounting for a mean capture rate of 1.67 sandflies per trap per night. This species demonstrated minimal activity across other months and was notably absent in January and July, coinciding with the peak dry and rainy seasons, respectively.

Species with low detection frequencies, such as *Se. logonensis* and *Se. affinis vorax*, were observed in only one month and excluded from further analysis due to their limited density.

Diversity indices of sandflies collected in Kousseri according to months

Table 4 presents the diversity indices of sandflies collected in Kousseri from September 2020 to August 2021. Species richness varied from six to nine species across the captured months. The highest species richness (S = 9 species)

Table 4. Alpha diversity indices of female sand ly collected in Kousseri between September 2020 and August 2021 at temporal scale.

	Collection months											
Diversity indices	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Abundance	75	50	62	124	394	238	232	191	200	206	159	219
Richness (S)	8	6	8	7	8	9	9	9	8	9	7	9
Shannon-Weiner (H')	1.56	1.39	1.27	1.43	1.22	1.64	1.59	1.45	1.72	1.62	1.84	1.41
Simpson (1-D)	0.75	0.67	0.59	0.65	0.56	0.74	0.73	0.68	0.78	0.75	0.83	0.67
Evenness (J)	0.8	0.78	0.61	0.74	0.59	0.75	0.72	0.66	0.83	0.74	0.95	0.64

was recorded from February to August, excluding July (S = 7 species) and May (S = 8 species) while the lowest species richness was observed in October, with only six species out of the eleven species encountered. The highest values of Shannon diversity (H'), Simpson dominance (1-D) and Evenness (E) indices were recorded in July and May (H'=1.84; 1-D = 0.83; J = 0.95 and H'=1.72; 1-D = 0.78; J = 0.83, respectively), indicating an even distribution of species. Conversely, the lowest values of these indices were observed in January and November (H'=1.22; 1-D = 0.56; J = 0.59 and H'= 1.27; 1-D = 0.59; J = 0.61, respectively), due to the strong dominance of *Se. antennata* over other sandfly species.

Fig. 3 presents a non-metric multidimensional scaling (NMDS) ordination, based on Bray-Curtis dissimilarities, highlighting monthly patterns in sandfly abundance during dry and rainy months. Red and black circles represent monthly samples from the dry and rainy seasons, respectively. The stress value of 0.1 indicates a reliable representation of the multivariate data in two dimensions. The ordination reveals distinct seasonal clustering. September, December and March are positioned to the far right of the NMDS1 axis and below NMDS2, suggesting a degree of similarity in species composition and abundance. September marks the end of the rainy season and the onset of drier conditions, potentially driving transitional shifts in species composition. October and November, located to the

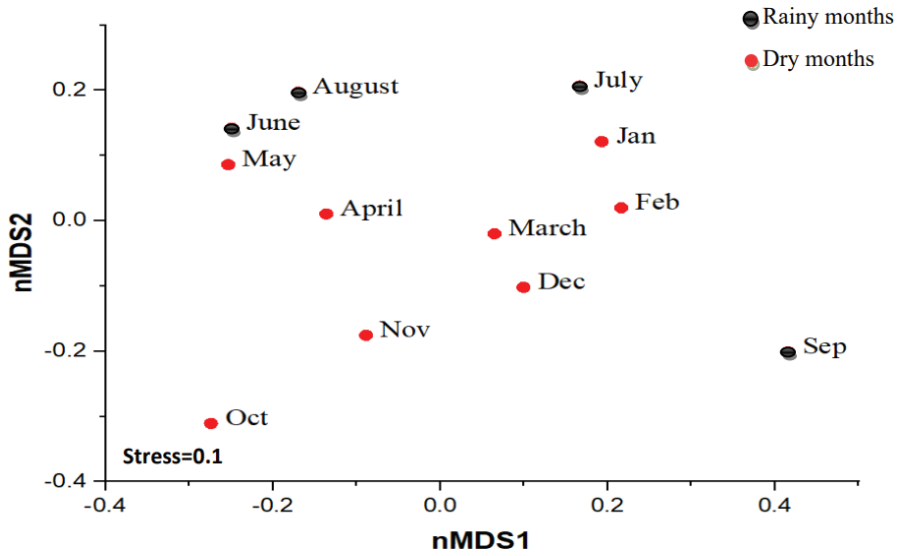

Figure 3. A Non-Metric Multidimensional Scaling (NMDS) plot on Bray-Curtis dissimilarities showing monthly variation in sandfly species abundance (Red = Dry season, Black = Rainy season).

left of NMDS1 and below NMDS2, exhibit a distinct pattern from other months, possibly reflecting environmental stabilisation towards dry-season conditions. January, February and July show relatively similar ordination positions, indicating comparable species abundance profiles. Notably, July falls in the middle of the rainy season, suggesting ongoing ecological changes. Finally, April, May, June and August are grouped to the far left of NMDS1 and above NMDS2, implying shared species abundance characteristics. This cluster may reflect peak rainy-season dynamics, with environmental factors favouring specific sandfly assemblages.

Discussion

This study established that the phlebotomine sandflies from Kousseri belonged to ten *Sergentomyia* and only one *Phlebotomus* species. In a previous study in Kousseri, it was recognised that *Phlebotomus* genus was represented by only one species, *Ph.* (*Phlebotomus*) *duboscqi* (Neveu-Lemaire) (Rageau, 1951), which is the only proven vector of human CL caused by *Leishmania major* (Dondji et al. 1998; Ngouateu et al. 2012) and suspected vector of VL (Tateng et al. 2018). However, some species from the genus *Sergentomyia* including *Se. schwetzi* have been reported to transmit canine leishmaniasis (Senghor et al. 2016).

Sandfly abundance has been shown to be influenced by a number of micro-environmental elements, such as the presence of animals, shaded vegetation (trees and agricultural fields), living conditions and husbandry activities (Risueño et al. 2017). The higher sandfly abundance observed in rural areas compared to suburban and urban locations aligns with the findings of Amira et al. (2022) in northern Algeria and Jalali et al. (2022) in Jajarm County, North Khorasan, Iran, who reported high sandfly abundance in rural regions. However, these data contrast with Tateng et al. (2023), who reported increased sandfly abundance in peri-urban settings. In the rural and suburban ecotopes of Kousseri, dwellings mainly consist of unplastered mud walls, which create crevices that may serve as shelters for adult sandflies, providing resting, breeding and mating sites (Elnaiem et al. 1999). Additionally, the presence of vegetation (mainly rice paddies) and animals near the compounds in rural ecotope may provide both sugar as an energy source and blood meals for egg development during adult female oviposition (Cecílio et al. 2022). This ecological pattern enhances contact between sandflies, domestic animals and people. However, the low number of sandflies captured in urban areas is consistent with the findings of Senne et al. (2021), who also reported reduced sandfly numbers in urban locations. The urban area surveyed in this study was predominantly residential and characterised by dense human occupation with a good level of urbanisation, featuring both traditional and generally modern plastered houses. This suggests that plastering walls of human residences and animal shelters may be effective in reducing the sandfly population as supported by Kumar et al. (1995).

Data gathered from this study showed that *Se. distincta* proportions decreased from urban to rural areas, suggesting a preference for densely populated regions where they may primarily feed on humans. Similar patterns were reported by Tateng et al. (2023), highlighting that this sandfly species, along with *Se. affinis vorax* and *Se. schwetzi*, likely exploit human and farmyard animals' blood meals, reflecting their reduced relative abundance from town to sylvatic areas. Despite the lack of evidence that *Sergentomyia distincta* may feed on humans, sandflies

from this genus are primarily known for their preference for cold-blooded animals, mainly small reptiles including lizards and geckos (Lewis and Dyce 1988), which may be found in dwellings in Kousseri. *Sergentomyia* sandflies are traditionally involved in the transmission of reptile *Leishmania* (Krüger et al. 2023). However, studies have suggested that some species could be implicated in the transmission of human pathogenic *Leishmania* (Maia and Depaquit 2016; Senghor et al. 2016; González et al. 2020). Further investigation is needed to assess the vectorial capacity of *Sergentomyia* sandflies, their feeding behaviour and their potential contribution to *Leishmania* spp. epidemiology in the country. Determining the blood-meal sources of this species would provide a better understanding of why they prefer urbanised, densely populated human dwellings.

Notably, our study identified *Se. schwetzi* as the second most abundant species, predominantly found in peri-urban ecotopes. This finding indicates that this sandfly species prefers peri-urban areas where animals' shelters are nearby humans' dwellings with an irregular garbage collection and existence of natural vegetation, mainly trees. However, *Se. schwetzi* may feed on both people and animals. It is important to note that *Se. schwetzi* has been suspected to transmit *Leishmania infantum* (Nicolle, 1908), the causative agent of canine leishmaniasis (Senghor et al. 2016). The exclusive occurrence of *Se. affinis vorax* in the peri-urban area, despite its relative low proportion, could indicate a level of resilience and adaptability that allows it to thrive in peri-urban settings. This finding differs from Tateng et al. (2023) who reported *Se. affinis vorax* as one of the most abundant species in the Mokolo CL focus. Most importantly, the number of *Se. antennata*, *Se. clydei*, *Se. adleri*, *Se. africana* and *Se. squamipleuris* populations decreased from rural to urban areas. A rural ecotope might provide more favourable ecological conditions, such as appropriate microclimates and vegetation cover mainly rice paddies, which are essential for the survival and reproduction of these species. This result diverges from Tateng et al. (2023), who recorded high proportions of *Se. antennata*, *Se. squamipleuris* and *Se. africana* from the sylvatic environment and relatively abundant *Se. clydei* and *Se. adleri* in the suburban area. Understanding why these sandfly species prefer a rural area over peri-urban and urban settlements is critical to designing effective management tactics to limit the risk of disease transmission. Interestingly, the high prevalence of *Ph. duboscqi* in peri-urban and rural areas compared to urban sites suggests a preference for environments with animal sheds and cracked walls and floors, which are typically found in peri-urban and rural areas. Is it worth noting that *Ph. duboscqi* is a generalist vector of *Leishmania major* in the OW, indicating that host selection is primarily a matter of availability, specifically, host number and size rather than preference (Bongiorno et al. 2003).

Sandfly species are highly adaptive to temperature and humidity ranges around 25–28 °C and saturated humidity (Israël et al. 2022). These conditions are like the Sudano-Sahelian climate type of Kousseri and may explain the year-round presence of sandfly in the area, but several other abiotic (latitude, surface water and wind) and biotic factors (vegetation, host species) may also influence sandfly distribution. Reportedly, climatic and environmental conditions of temperature, humidity and rainfall affect the monthly abundance and the distribution of sandfly (Chaniotis et al. 1971; Lane 1993; Rohr et al. 2011; Falcao de Oliveira et al. 2016). These factors are known to affect survival rates and population sizes (Fouque and Reeder 2019) which, in turn, affect the ep-

idemiology and dynamics of pathogen transmission to the human population (Rohr et al. 2011). An understanding of monthly distribution of sandflies associated with climatic factors such as temperature, rainfall and relative humidity can contribute to a better comprehension of the occurrence of phlebotomine sandflies and their population density in Kousseri.

The higher occurrence of sandflies during the dry season, especially during the months of January and March, contradicts Dondji et al. (2000) and Tateng et al. (2023) in the Mokolo CL focus where higher numbers of sandflies were found during the rainy season. This seasonal divergence is further highlighted by studies from north-eastern Brazil, where most of the collected sandfly species exhibited rainfall and humidity driven dynamics (Pinheiro et al. 2021). However, our results are consistent with those from arid and semi-arid regions, such as Iran (Jalali et al. 2022) and parts of Kenya, where sandfly populations peaked during the dry season, likely due to optimal microclimatic conditions and habitat suitability (Hassaballa et al. 2021). The lowest abundance of sandflies recorded in September and October representing the rainy season and the beginning of the dry season, respectively was expected because, during these periods, there was flooding due to excessive water. Unlike mosquitoes, the life cycle of the sandfly is fully terrestrial and does not require water, but sufficient moisture is needed in the soil (Cecílio et al. 2022). The higher number of female sandflies than males in six of the twelve surveyed months, including September, October, January, April, June and July in this study, suggest a higher risk vector exposure during these months. This current result could be explained by the type of traps used rather than the disproportion in the natural sex-ratio of sandflies captured. Since sandflies are weak flyers, the presence of both males and females provides evidence of the presence of sandflies' breeding sites around human habitations. A further assessment of related sandfly activities and seasonal dynamics with host animals and human exposure patterns is necessary. This knowledge will be instrumental in designing more precise and sustainable vector control programmes.

The results of the current study found that *Se. antennata*, *Se. distincta*, *Se. squamipleuris* and *Se. schwetzi* were collected throughout the collection period, while *Se. africana*, *Se. clydei*, *Se. adleri*, *Ph. duboscqi* and *Se. inermis* were absent for at least one month. An earlier study on the distribution of sandflies in Mokolo found *Se. antennata*, *Se. schwetzi* and *Se. africana* to be present every month (Dondji et al. 2000). This result may explain their high adaptability in the country. Indeed, these sandfly species have a wide range of distribution in Afro-tropical areas (Lane 1993; Akhoundi et al. 2016).

The observed unimodal distribution trend of *Se. antennata* peaking in January during the middle dry season and a trimodal distribution trend of *Se. schwetzi* with the most important peak in September, differ with the findings of Dondji et al. (2000) who showed a bimodal peaks pattern for *Se. antennata* in April and October and a unimodal distribution trend for *Se. schwetzi* in October. The presence of *Se. africana* in all months, except in October with a peak in March (4.86 sandflies per light trap per night) during the dry season differs from Dondji et al. (2000) who found this species decreasing from January to December in Mokolo with no collection in August and November. These discrepancies emphasise the role of localised environmental factors in shaping species dynamics.

Even though some *Sergentomyia* sandfly species were present in every month, the monthly distribution of *Ph. duboscqi* in Kousseri reveals a unimodal peak in September with absences observed in January during the dry season and in July during the rainy season. Our result differ from Tateng et al. (2019) who found a dominant peak of this species in July in Mokolo, which correspond to the annual CL case peak in the Mokolo outbreak between September and October (Ngouateu et al. 2012). Interestingly, our results are similar to those from Talbi et al. (2022), who reported unimodal trends in medically important species, including *Ph. sergenti* and *Ph. papatasi*, in Morocco. The population density of *Ph. duboscqi* in Africa vary seasonally along with the activity cycles of their rodent hosts (Munstermann 2019). With regard to this aspect, further research exploring reservoir host impacts on these fluctuations will be crucial, particularly given the zoonotic nature of some leishmaniasis.

While this study provides valuable baseline data on the monthly distribution of female phlebotomine sandflies in Kousseri, its limited one-year duration constrains the ability to discern consistent seasonal patterns and interannual variability. Extended longitudinal monitoring is imperative to elucidate the ecological drivers of sandfly population dynamics and to more precisely assess the influence of climatic and environmental factors on vector abundance and distribution. Such data are critical for providing information for the development of targeted and sustainable vector control strategies, as well as for refining predictive models of leishmaniasis transmission risk in endemic settings. Moreover, further investigation into key parameters of vectorial capacity, particularly anthropophilic behaviour and natural infection rates with *Leishmania* species, is essential to deepen our understanding of transmission dynamics and to reinforce epidemiological surveillance frameworks. These efforts will contribute to a more comprehensive and evidence-based approach to leishmaniasis prevention and control.

Conclusion

This study provides important insights into the occurrence and monthly distribution of sandflies in Kousseri. Our survey suggests that the presence of animal shelters around dwellings, vegetation (mainly paddy rice) and the housing conditions characterised by unplastered, cracked mud walls significantly contribute to the proliferation of sandfly populations, especially in rural settings. Although sandflies were active throughout the year, their abundance peaked during the dry season, notably in January and March. However, *Phlebotomus duboscqi*, the only species identified with proven vectorial competence for leishmaniasis in the region, was detected at very low densities and was not consistently present during the study period. Surveillance and intervention efforts should particularly target rural areas and the months when sandflies are more prevalent, such as January and March. However, this study faced several limitations including a limited timeframe (September 2020 to August 2021) and misidentification of male sandflies which could lead to inaccurate conclusions regarding species-specific monthly distribution patterns. Additionally, the study did not account for all environmental variables that could influence the monthly dynamics of sandfly populations, such as microclimatic conditions, vegetation cover and human activities. To address these gaps, future research should adopt a longitudinal design encompassing multiple years, identify male

sandflies and incorporate a broader array of ecological and climatic parameters. Such comprehensive approaches are essential for advancing our understanding of sandfly population dynamics and for providing information for evidence-based, sustainable strategies for leishmaniasis prevention and control in Kousseri and similar endemic regions.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Tebo Nzesseu Thierry Rostand: Methodology, Formal analysis, Resources, Investigation, Data Curation, writing original draft, Review and Editing; Tateng Ngouateu Aime: Conceptualisation, Methodology, Formal analysis, Investigation, Data Curation, Writing original draft, Review and Editing, Visualisation, Funding acquisition; Ngouateu Omer Bebe: Conceptualisation, Methodology, Investigation, Funding acquisition; Foyet Juluis Visnel: Formal analysis, Data Curation, Methodology, Ngnindji-Youdje Yannick: Methodology, Data curation; Khan Payne Vincent: Methodology, Supervision; Ndo Cyrille: Conceptualisation, Resources, Supervision; Dondji Blaise: Conceptualisation, Resources, Investigation, Writing – Original Draft, Review and Editing, Visualisation, Supervision, Project administration, Funding acquisition.

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Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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