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Land use and cover changes and sand fly (Diptera: Psychodidae) assemblages in an emerging focus of leishmaniasis

Mudança no uso e ocupação do solo e assembleias de flebotomíneos (Diptera, Psychodidae) em um foco emergente de leishmanioses

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Abstract

Land use and cover changes lead to fragmentation of the natural habitats of sand flies and modify the epidemiological profile of leishmaniasis. This process contributes to the infestation of adjacent rural settlements by vector sand fly species with different degrees of adaptation, promoting leishmaniasis outbreaks. This study aimed to assess land use and cover changes over a 12-year period and investigate the diversity and abundance of sand fly assemblages in the rural area of Codó, Maranhão State, Brazil. Temporal analysis of land use and cover changes was conducted using Sentinel-2 satellite imagery treated in QGIS software (free version 3.10) and classified using Orfeo Toolbox. Sand flies were sampled in alternate months between August 2022 and June 2023 using Centers for Disease Control and Prevention (CDC) light traps and white and black Shannon traps installed in peridomestic and extradomestic environments. Map images showed that the predominant land covers in 2012 and 2014 were dense and sparse vegetation, with few buildings. In 2021 and 2023, areas of sparse and dense vegetation were fragmented as

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new settlements were established. The entomological survey resulted in the capture of 3375 sand flies (CDC = 856, white Shannon = 650, black Shannon = 1969) belonging to 20 species. The most abundant species were *Psychodopygus wellcomei* Fraiha, Shaw & Lainson, 1971 (78.19%), followed by *Nyssomyia whitmani* (Antunes & Coutinho, 1939) (7.53%). Additionally, *Ny. whitmani* was the most abundant species (84.97%) in peridomestic environments, whereas *Ps. wellcomei* was the most abundant species (96.51%) in extradomestic environments. The sand fly assemblage was highly diverse, with a high abundance of competent vectors of *Leishmania* spp. These findings can promote community participation in surveillance and control efforts to prevent leishmaniasis cases.

KEYWORDS
anthropic action, ecology, *Leishmania*, Phlebotominae, timescale analysis, vectors

INTRODUCTION

Sand flies (Diptera: Psychodidae) are small insects that are widely distributed across the globe. Of the 1060 described taxa, 555 occur in the Americas and 279 in Brazil. Representatives are found across all Brazilian states, with a high diversity in the Amazon and Northeast forest ecosystems (Aguiar & Vieira, 2018; Galati & Rodrigues, 2023; Rebêlo et al., 2010; Rodrigues & Galati, 2024).

Given the hematophagous nature of female sand flies, some species are involved in the transmission of *Leishmania* protozoans, Ross, 1903 spp. (Kinetoplastida: Trypanosomatidae) to mammalian hosts, including humans (Akhoundi et al., 2016; BRASIL, 2017). These protozoa are the causative agents of visceral (VL) and cutaneous (CL) leishmaniasis, which, depending on the parasite species, can present with mild or severe clinical forms. These diseases represent a serious public health problem in tropical and subtropical countries, affecting millions of people every year (BRASIL, 2017; OPS, 2023).

A total of 40,170 cases of CL and 4306 cases of VL were reported in Brazil between 2020 and 2022. The former is most frequent in the Northern region (19,875 cases), whereas the latter is most common in the Northeast (2465 cases). Maranhão, a state located between the two regions, had the highest number of both VL (803) and CL (3604) cases in the same period (SINAN/SVS/MS, 2022). Given its geographical position, Maranhão encompasses areas of the Amazon biome and forest ecosystems that transition into the Cerrado biome, characteristics that favour the diversity of sand fly species (Aguiar & Vieira, 2018; Rebêlo et al., 2010).

A recent study examined the spatio-temporal dynamics of CL in Maranhão from 2007 to 2020. The findings revealed that CL was previously concentrated in the western part of the state and, over the years, spread to other regions, particularly to the north and east. This spread was associated with anthropic changes related to rural activities, such as the formation of new settlements, subsistence agriculture and livestock farming (De Sousa Oliveira et al., 2024). Several studies provided evidence that human-related actions, such as deforestation for agriculture, mining, logging, road works and the construction of urban environments, alter the natural cycle of the disease. The spread of leishmaniasis is often linked to environmental problems and anthropic actions that exert negative effects on vegetation cover, reducing the availability of food resources. As a result, vector and reservoir species migrate and become adapted to urbanised and anthropized environments, increasing the number of reported cases (Maroli et al., 2013; Moraes et al., 2020; Walsh et al., 1993).

The current study focuses on the municipality of Codó, where both forms of leishmaniasis are endemic. From 2020 to 2024, Codó had 36 VL cases and 65 CL cases, according to data from the Municipal Department of Endemics. The first entomological surveys recorded 11 sand fly species (Rebêlo et al., 1999; Silva et al., 2015). In the cited studies, *Lutzomyia longipalpis* (Lutz and Neiva, 1912), a local vector of *Leishmania* (*Leishmania*) *infantum* Cunha & Chagas, 1937, the etiological agent of VL, was found in peridomestic environments associated with domestic animals and livestock, such as chicken coops in peri-urban areas. On the other hand, *Nyssomyia whitmani* (Antunes and Coutinho, 1939), a competent vector for *Leishmania* (*Viannia*) *shawi* Lainson et al., 1989 and *L.* (*V.*) *braziliensis* Vianna, 1911, was predominantly found in rural areas, followed by urban areas.

Understanding the assemblage of vector sand flies is crucial for the epidemiological surveillance of leishmaniasis. The study of entomological and ecological factors, combined with geospatial technologies, such as remote sensing, allows the detection of environmental changes and the visualisation of the distribution of sand flies in a given region. This information facilitates the delimitation of geographical areas with a potential risk of leishmaniasis transmission (Hugh-Jones, 1989; Machault et al., 2011).

As a result of population growth, Codó has undergone intense changes in land use and cover over the years. Common activities include the removal of vegetation cover for the construction of large popular housing complexes, access roads and agropastoral systems in urbanised outskirts. The hypothesis raised in this study is that land use and cover changes contribute significantly to the proliferation of vectors adapted to anthropized environments. In view of the endemic status of CL and VL in Codó, it is believed that the most successful vectors in this municipality, as demonstrated by Rebêlo et al. (1999) and Silva et al. (2015), continue to be *Lu. longipalpis* and *Ny. whitmani*, which are also the most important vectors of leishmaniasis in Maranhão State (Rebêlo et al., 2010). To test this hypothesis, an entomological survey was conducted in a rural area where anthropogenic interventions have resulted in forest fragments. The aims were to assess land use and record changes over a 12-year period and

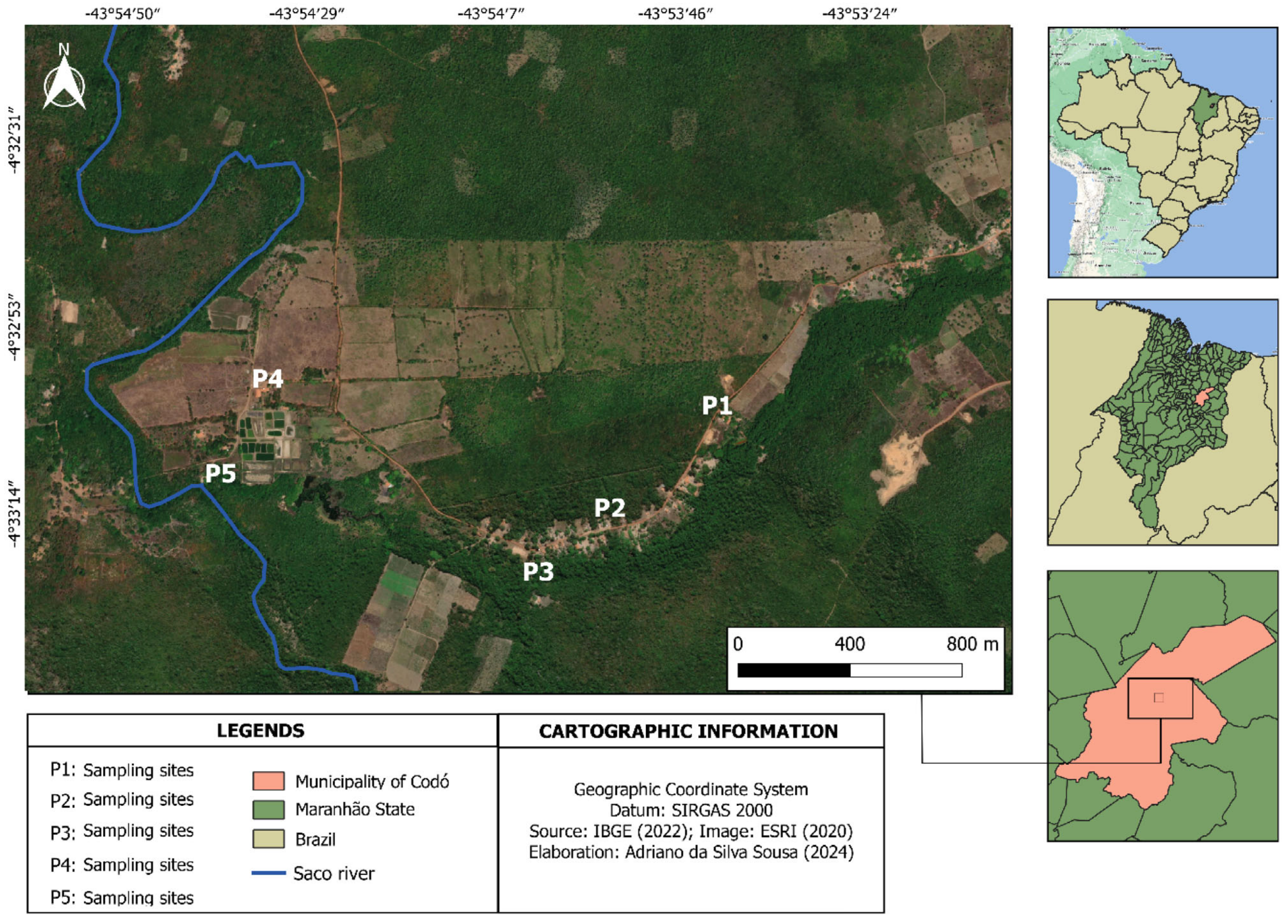

FIGURE 1 Location of sand fly sampling points where traps were installed in the Santana IV rural settlement, Codó, Maranhão State, Brazil.

investigate the ecological aspects of sand fly assemblages and the risk of leishmaniasis transmission.

MATERIALS AND METHODS

Study area

This study was conducted in the Santana IV rural settlement, Codó (04°27′12.8″ S 43°53′01.7″ W), Eastern Mesoregion of Maranhão State, Brazil (Figure 1). The municipality has an area of 4364.5 km^2 and an estimated population of 114,275 people, with a population density of 26.20 people/km^2 (IBGE, 2022). Vegetation cover varies according to relief characteristics, proximity to watercourses and the extent of anthropic transformations. The predominant vegetation type is open forest/babassu forest, occupying the entire valley of the Itapecuru River. The main tree species are babassu palm (*Attalea speciosa* Mart. ex Spreng.) and carnauba [*Copernicia prunifera* (Miller) H.E.Moore]. Another common type of vegetation cover is *campo cerrado*, found mainly in the east, north-west and south-west parts of the municipality (Correia Filho et al., 2011).

The climate is semi-humid, transitioning to semi-arid with precipitation. According to the Köppen classification, the climate is of the Aw type, with rainy summers and dry winters. The driest month has less than 60 mm rainfall. Temperatures in the coldest month remain above 18°C. The annual average temperature is about 27°C, and the maximum temperature is 36°C. Air humidity reaches high values during the rainy season, indicating that this parameter is directly related to the rainfall regime of the region. The average annual rainfall is approximately 1200 mm, with the wettest quarter being January, February and March (Correia Filho et al., 2011; Lima, 1998).

Five sampling points were located in peridomestic environments (Animal shelter backyard): P1 (04°55′062″ S 043°89′450″ W), P2 (04°55′402″ S 043°89′822″ W), P3 (04°55′474″ S 043°90′048″ W), P4 (04°54′959″ S 043°90′936″ W) and P5 (04°55′161″ S 043°91′053″ W). These areas were close to houses, debris and waste. The soil was moist, and various domestic animals were observed, such as dogs, cats, pigs, cattle, chickens, guineafowl (*capotes*), horses and geese. Samplings were also performed in extradomestic environments located at least 500 m away from peridomestic sampling points. Extradomestic environments were characterised by primary forests (fragmented) with the presence of fruit trees (cashew and mango trees) and shaded soil, with the presence of wet litter and bogs in the vicinity of the Saco River.

Sand fly collection and identification

Sand flies were captured at the five sampling points (P1–P5) in the dry (August–December 2022) and wet (February–June 2023) periods. In alternate months, two Centers for Disease Control and Prevention (CDC) light traps were installed per sampling point, one in a peridomestic environment (near residences where animals are raised) and another in an extradomestic environment (within a fragment of closed vegetation), totalling 10 traps. Traps were kept in the field for two consecutive nights, being installed at 18:00 h and removed the next day at 6:00 h. Active sampling was performed from 18:00 to 21:00 h using white and black Shannon traps (Galati et al., 2001).

Captured sand flies were taken to the Medical Entomology Laboratory, Caxias Campus, Maranhão State University (UEMA), for sex discrimination, clearing and dissection. All collected sand flies (males and females) were dissected by removing the head, thorax and the last three segments of the abdomen and mounted on a glass slide with Canada balsam for species identification. The rest of the body was individually stored dry at -20°C in a 1.5-mL tube for future molecular studies. Species were identified using the updated version of the classification system proposed by Galati (2024). Genus abbreviations follow Marcondes (2007).

Data analysis

For the timescale analysis of land use and cover in the Santana IV settlement, Sentinel 2 satellite imagery was acquired over sand fly sampling points in 2012, 2014, 2021 and 2023. Images were downloaded from the LandView website. The analysed years were chosen because they were within a 12-year time frame. Image treatment for visualisation of landscape changes was carried out using Quantum GIS (QGIS) software free version 3.10 based on image features and characteristics. Shapefile files were obtained from the Brazilian Institute of Geography and Statistics (IBGE) database to delimit sampling points. Orfeo Toolbox, an open source project native to QGIS for state-of-the-art remote sensing, was used to classify images. Developed by the open-source geospatial community, Orfeo Toolbox can process high-resolution optical, multispectral and radar images on a terabyte scale.

For the analysis of sand fly assemblages, relative abundance was estimated as a percentage of the total number of collected individuals. Species richness was estimated from the number of identified species. The abundance and diversity of sand flies captured in the dry and wet seasons in peridomestic and extradomestic environments were measured by the Shannon diversity index (SHDI). Cluster analysis was performed to compare the composition of sand fly populations between dry and wet periods based on the presence and absence of species, using Jaccard's similarity index (Ludwig & Reynolds, 1988). Rarefaction curves for the dry and wet periods were constructed by extrapolating the expected number of species to larger sample sizes. These curves were used to estimate species richness in each period and compare the completeness of samples. The effects of temperature, relative humidity, peridomestic conditions and extradomestic conditions on abundance and diversity were examined using generalised linear mixed models (GLMMs) at the 5% significance level. Temperature (°C) and relative humidity (%) were measured using a digital thermohygrometer (IHT-2200, Instrutemp) at each sampling point when traps were installed and removed. The constancy index (CI) was calculated by the equation $CI = P \times 100/N$, where P is the number of collections in which the species was present and N is the total number of months in which the species was collected. CI values were used to group species into three categories: constant (CI ≥50%), accessory (25% < CI < 50%) and accidental (CI ≤25%) (Silveira-Neto et al., 1976). Data analysis was conducted using specific packages for R software version 4.3.1 (2023).

RESULTS

Using the images acquired from the spatial analysis website LandView, we were able to identify and characterise the dynamics of land use and cover in Santana IV in 2012, 2014, 2021 and 2023 (Figure 2). The following classes were used for land cover characterisation: buildings, dense vegetation, sparse vegetation, pasture/plantation and bare soil. These classes allowed an evolutionary analysis of land use and cover, indicating the factors that contribute to the presence or permanence of sand flies near human settlements in the study area. In 2012 and 2014, there was a predominance of dense and sparse vegetation, few areas with bare soil and few buildings. In 2021 and 2023, sparse vegetation increased and dense vegetation underwent fragmentation as new settlements were built. Such transformations were evident at P4, which, in the first two years of analysis, consisted exclusively of bare soil. As of 2021, P4 was characterised by the presence of buildings (Figure 2).

The entomological survey resulted in the capture of 797 sand fly specimens (1:1 males/females) using CDC traps and 2508 specimens (1:3 males/females) using Shannon traps, resulting in a total of 3305 individuals. The sample was distributed into four subtribes and 10 genera. The most abundant species were *Psychodopygus wellcomei* Fraiha, Shaw, & Lainson, 1971 (79.85%) and *Ny. whitmani* (7.69%). The other identified species had a relative abundance of less than 5% (Table 1).

Species abundance differed according to the collection method. With CDC traps, six species were the most abundant, namely *Ps. wellcomei* (28.73%), *Ny. whitmani* (24.22%), *Brumptomyia brumpti* (Larousse, 1920) (12.05%), *Evandromyia lenti* (Mangabeira, 1938) (9.41%), *Ev. evandroi* (Costa Lima & Antunes, 1936) (7.65%) and *Ny. antunesi* (Coutinho, 1939) (5.77%). The other identified species had an abundance lower than 5%. In the sample captured using Shannon traps of both colours, *Ps. wellcomei* (96.09%) was the most abundant species. Additionally, for collections made using Shannon traps of both colours, sand fly abundance was much higher in the wet period (February–June 2023) (Figure 3a). However, diversity (SHDI) was higher in the dry period (August–December 2022) (Figure 3b). All 20 identified species were captured with CDC traps, whereas only 12 of the 20 identified species were captured with the white Shannon trap and 8 were captured with the black Shannon trap (Table 1).

Sand flies captured in the extradomestic environment were more abundant (57.94%, $n = 496$), with a predominance of *Ps. wellcomei* (96.51%, $n = 229$) and *Psathyromyia hermanlenti* (Martins, Silva & Falcão, 1970) (83.33%, $n = 12$). In the peridomestic environment, the most abundant species were *Ny. whitmani* (84.97%, $n = 193$), *Sciopemyia sordellii* (Shannon & Del Ponte, 1927) (75%, $n = 12$) and *Lu.*

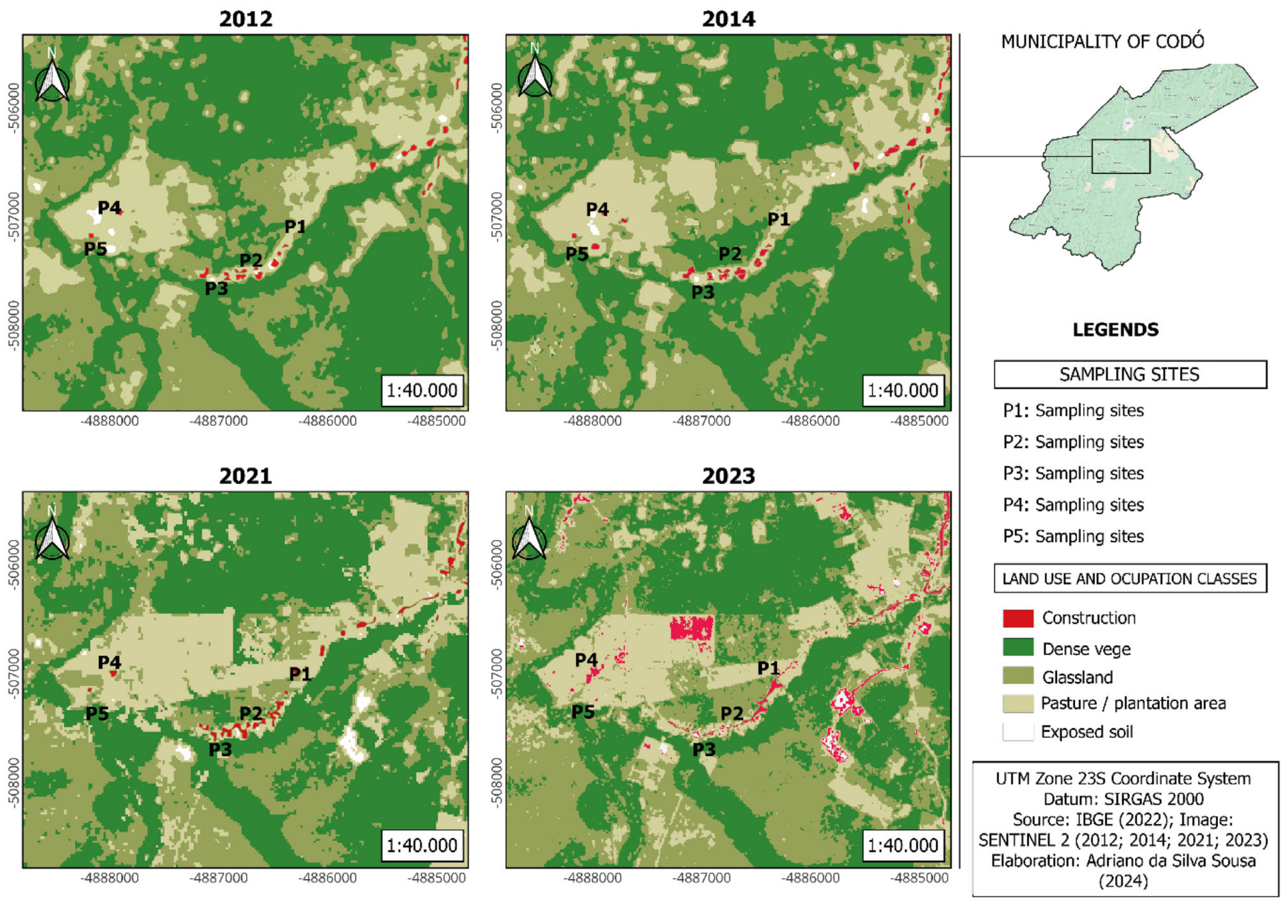

FIGURE 2 Changes in vegetation cover and land use throughout 2012, 2014, 2021 and 2023 in the Santana IV rural settlement, Codó, Maranhão State, Brazil.

gomezi (Nitzulescu, 1931) (83.33%, $n = 6$). The species *Lu. longipalpis* and *Ny. intermedia* (Lutz & Neiva, 1912) occurred only in the peridomestic environment. *Evandromyia termitophila* (Martins, Falcão, & Silva, 1964) and *Micropygomyia peresi* (Mangabeira, 1942) occurred only in the extradomestic environment (Figure 4a). In both types of environments, sand fly abundance was higher in the wet period (Figure 3b). However, in the peridomestic environment, SHDI was higher in the dry period (Figure 4c).

The species *Br. brumpti*, *Ny. whitmani* and *Ev. evandroi* were captured in all months of sampling and classified as constant (CI = 100%). The species *Lu. longipalpis*, *Mi. trinidadensis*, *Psathyromyia aragaoi* (Costa Lima, 1932) and *Viannamyia furcata* (Mangabeira, 1941) were considered accessory (CI = 33.33%). *Micropygomyia peresi* was collected in June and *Ny. intermedia* only in April, and both were classified as accidental (CI = 16.66%) (Figure 5a). The vector species *Ps. wellcomei* did not appear in August or October (dry period), having low relative abundance in December (1%). *Psychodopygus wellcomei* (76.51%), together with the vector *Ny. whitmani* (34.85%), had the highest relative abundance in April, coinciding with an increase in humidity (Figure 5a,b). However, considering the entire collection period, humidity ($p = 0.005647$) and temperature ($p = 0.006952$) had a negative correlation with sand fly abundance (Figure 5c,d).

In the wet season (February, April and June), 19 species were caught, except *Mi. trinidadensis* (Newstead, 1922). By contrast, 16 species were collected in the dry period (August, October and December), except *Lu. sherlocki* (Martins, Silva, & Falcão, 1971), *Mi. peresi*, *Ny. intermedia* and *Pa. aragaoi* (Figure 3a). In the cladogram, the dry and wet periods were placed in separate clusters, indicating distinct communities between periods, with a cophenetic coefficient of approximately 95%. However, this separation does not have an abrupt transition, given the continuous nature of the temporal data, with changes occurring gradually over time (Figure 6a).

Analysis of the rarefaction curve revealed a greater species diversity during the wet period. The curve generated for the dry period showed an inadequate rarefaction, due to the high abundance of *Ps. wellcomei* during the wet period. As a result, there was a reduction in sampling during the dry period. The shape of the rarefaction curve suggests that, for both periods, the sampling effort was sufficient to capture species diversity, as evidenced by the extrapolation trend (Figure 6b).

DISCUSSION

In the present study, the analysis of spatial maps revealed a noticeable change in vegetation cover over 12 years in the studied area. Changes occurred with the implementation of human activities, such as subsistence agriculture and construction of settlements. Activities that

TABLE 1 Caught sand flies by species and sex using CDC and Shannon traps in alternate months between August 2022 and June 2023 in Codó, Maranhão, Brazil.

		Capture method					
		CDC		Shannon			
				White	Black		Overall total
Subtribe	Species	♂/♀	N (%)	♂/♀	♂/♀	N (%)	N (%)
Brumptomyiina	*Br. brumpti*	61/35	96 (12,05)	0/2	0	2 (0,08)	98 (2,97)
Lutzomyiina	*Ev. evandroi*[a]	25/36	61 (7,65)	0/3	0/1	4 (0,16)	65 (1,97)
	Ev. lenti[a]	47/28	75 (9,41)	7/2	2/1	12 (0,48)	87 (2,63)
	Ev. saulensis (Floch & Abonnenc, 1944)	4/25	29 (3,64)	0	0	0 (0)	29 (0,88)
	Ev. termitophila	1/1	2 (0,25)	0/1	0	1 (0,04)	3 (0,09)
	Lu. sherlocki	1/1	2 (0,25)	1/3	1/2	7 (0,28)	9 (0,27)
	Lu. gomezi[a]	0/6	6 (0,75)	1/2	0	3 (0,12)	9 (0,27)
	Lu. longipalpis[a]	1/1	2 (0,25)	0	0	0 (0)	2 (0,06)
	Sc. sordellii	1/11	12 (1,51)	1/0	1/0	2 (0,08)	14 (0,42)
Psychodopygina	*Bi. flaviscutellata*[a] (Mangabeira, 1942)	0/12	12 (1,51)	0/1	0/1	2 (0,08)	14 (0,42)
	Ny. whitmani[a]	152/41	193 (24,22)	34/18	5/4	61 (2,43)	254 (7,69)
	Ny. intermedia[a]	0/1	1 (0,13)	0	0	0	1 (0,03)
	Ny. antunesi[a]	5/41	46 (5,77)	0	0	0	46 (1,39)
	Pa. hermanlenti	11/1	12 (1,51)	0	0	0	12 (0,36)
	Pa. bigeniculata (Floch & Abonnenc, 1941)	0/3	3 (0,38)	0/1	1/0	2 (0,08)	5 (0,15)
	Pa. aragaoi	2/6	8 (1,00)	0	0	0	8 (0,24)
	Ps. wellcomei[a]	63/166	229 (28,73)	67/395	547/1401	2410 (96,09)	2639 (79,85)
	Vi. furcata	0/4	4 (0,50)	0	0	0	4 (0,12)
Sergentomyiina	*Mi. trinidadensis*	0/2	2 (0,25)	0/2	0	2 (0,08)	4 (0,12)
	Mi. peresi	0/2	2 (0,25)	0	0	0	2 (0,06)
	TOTAL	374/423	797 (100)	111/430	557/1410	2508	3305
	Relative abundance %	46,93/54,07	-	21,57	78,43	-	-
	N° of species	20	-	12	8	-	-

Abbreviations: ♂/♀, absolute number of male/female; *N*, number of sand flies captured.
[a]Vector species or species suspected of transmitting *Leishmania* spp.

cause deforestation not only modify vegetation cover but also affect the structure of sand fly assemblages (Rebêlo et al., 2019).

Native vegetation is quite fragmented in the areas surrounding Santana IV. Nevertheless, the region harbours wild sand fly assemblages. Sand flies maintain contact with the settlement through fragmented primary vegetation that connect with the tree population of peridomestic environments. Domestic animals reared in peridomestic environments (e.g., birds, pigs, horses, cattle, dogs and cats) attract sand flies. With this, wild, peridomestic and domestic cycles interact, favouring the maintenance of sand fly populations near settlements (Figueiredo et al., 2017).

The most abundant sand flies recorded here, *Ps. wellcomei* and *Ny. whitmani*, hold great medical and veterinary importance, as they are involved in the transmission of etiological agents of CL (Rangel et al., 2018). Furthermore, the other identified species, namely *Ev. evandroi*, *Ev. lenti*, *Lu. gomezi*, *Lu. longipalpis*, *Bi. flaviscutellata*, *Ny. intermedia* and *Ny. antunesi*, may have epidemiological significance. Such species were implicated as vectors or suspected to transmit *Leishmania* spp. in several locations in Brazil, including endemic areas of the Northeast (Leonel et al., 2024; Rangel et al., 2018; Souza et al., 2016), hence the importance of continuous entomological monitoring in this area.

Sand fly species composition differed between wet and dry periods. Some species were restricted to the wet period, such as *Lu. sherlocki*, *Mi. peresi*, *Pa. aragaoi* and *Ny. intermedia*, the last of which is a vector of etiological agents of CL. Only *Mi. trinidadensis* was collected in the dry period. The presence of certain species in a specific period was also reported by Rigg et al. (2021), who observed that sand fly composition was influenced by temperature and humidity variations. Sand flies are susceptible to habitat changes, but this susceptibility varies according to species and may influence vector–parasite interactions (Teodoro et al., 1991; Travi et al., 2002). Seasonal variations can influence disease transmission by altering the dynamics of such interactions.

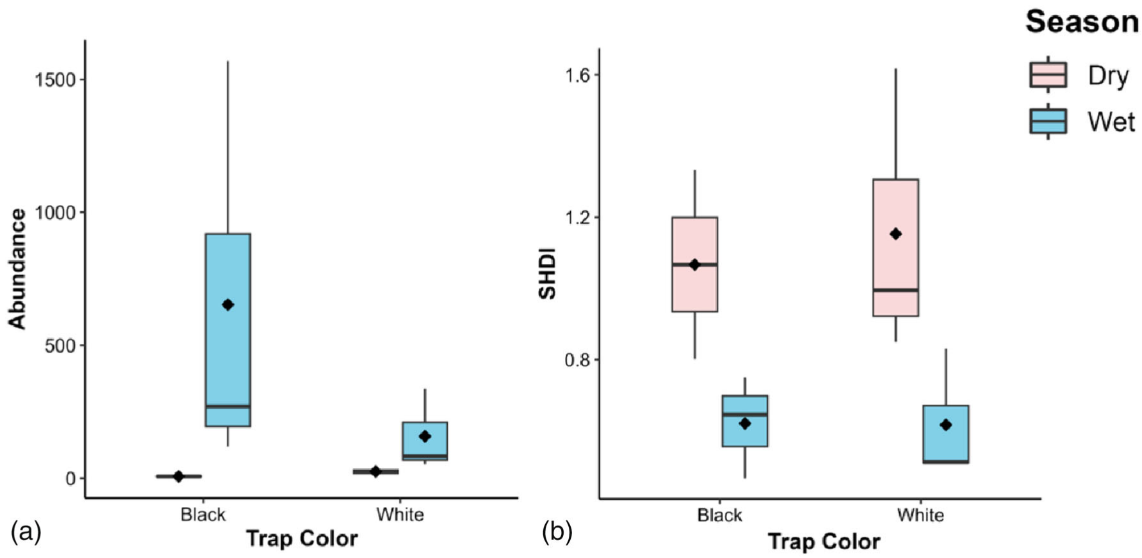

FIGURE 3 Sand fly abundance and diversity (SHDI) in Codó, Maranhão State, Brazil, as assessed using black and white Shannon traps in the dry (August–December 2022) and wet (February–June 2023) periods.

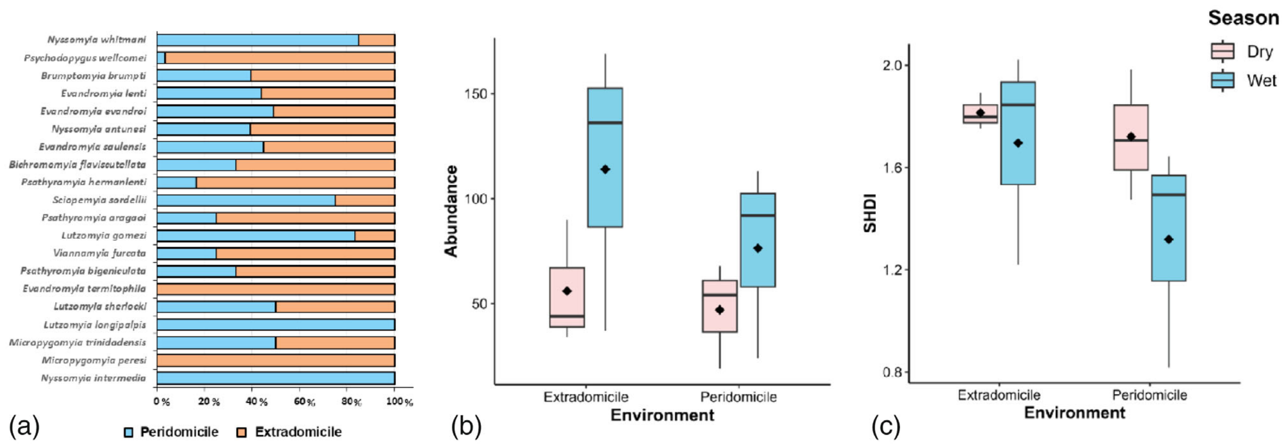

FIGURE 4 Distribution of sand fly species collected in extradomicile and peridomicile environments (a); abundance of sand flies according to the type of environment (extradomicile and peridomicile) and the dry and wet seasons (b); and Shannon diversity according to the type of environment and the dry and wet seasons (c). Caught sand flies using CDC traps in Codó, Maranhão, Brazil.

For the dry period, the species accumulation curve did not stabilise, due to the high abundance of *Ps. wellcomei* in the wet season. This finding may also indicate that new species may be collected with a greater sampling effort. Nevertheless, our sampling effort was sufficient to sample the diversity of species in the area in both dry and wet periods.

The high abundance of *Ps. wellcomei* deserves mention. This species has been reported as infrequent or occasional in virtually all studies conducted in Maranhão State. However, an increase in the abundance of sand flies in the wet season appears to be a consistent pattern in equatorial tropical areas. This phenomenon was reported in most studies conducted across Maranhão State, as observed here for *Ps. wellcomei* and *Ny. whitmani*, two important CL vectors. Continuous rains are conducive to the proliferation of sand flies, as humidity and food availability increase and temperatures decrease. During the wet season, it is common to observe a decrease in the number of insects collected in a given month, leaving the false impression of a reduction in population size. However, as has been discussed in various studies, heavy rains interfere with insect collection, impairing the displacement of adult insects and the efficiency of traps. Furthermore, rainstorms may damage breeding sites, eliminating immature forms (Martins et al., 2011; Moraes et al., 2015; Penha et al., 2013).

In the dry period, marked by increased temperatures, food resources become scarce and there is a decline in the abundance of sand fly populations. This phenomenon differs from that observed in subtropical areas, where insects enter diapause because of the

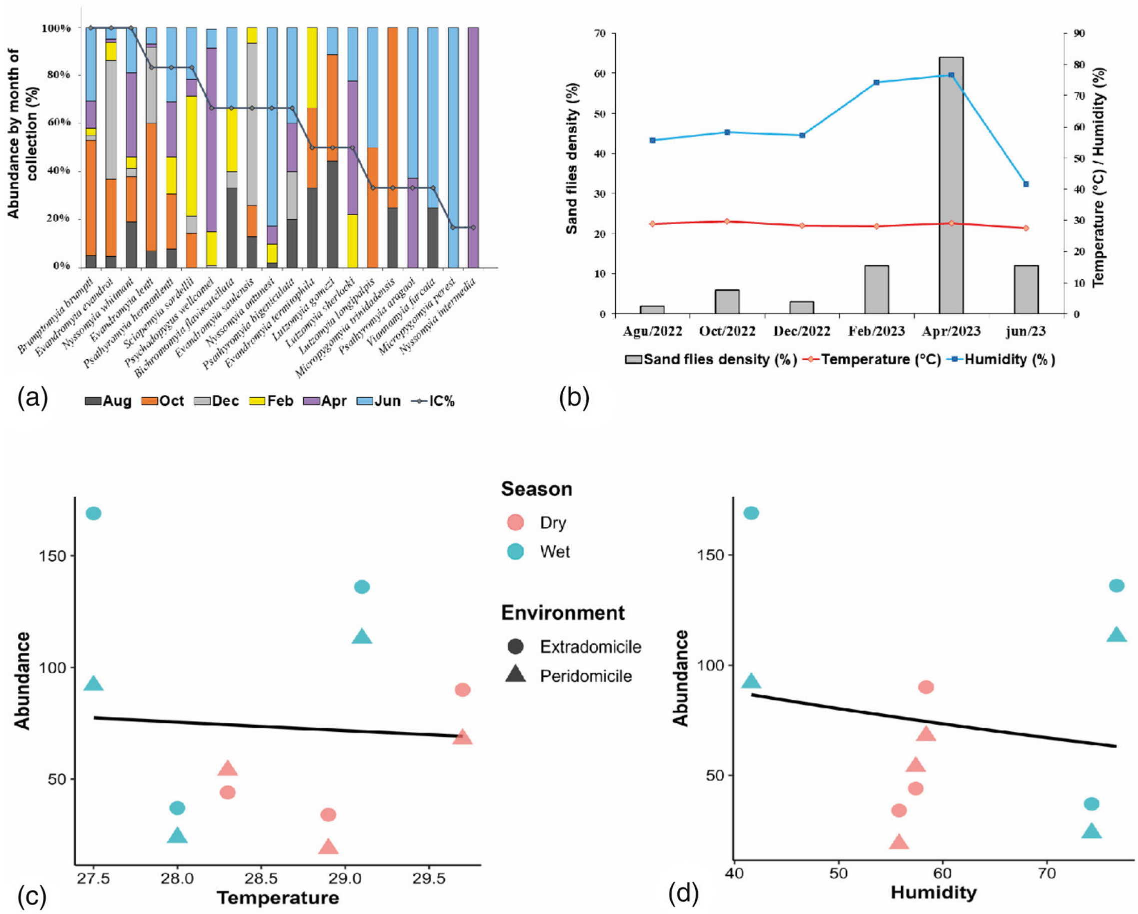

FIGURE 5 Monthly distribution of sand fly species and constancy index (CI) values (a); monthly distribution of climatic variables (temperature and humidity) and sand flies between August 2022 and June 2023 (b); correlation between sand fly abundance and temperature according to the type of seasons (dry and wet) and environment (extradomicile and peridomicile) (c); correlation between sand fly abundance and humidity according to the type of seasons (dry and wet) and environment (extradomicile and peridomicile) (d). Caught sand flies using CDC traps in Codó, Maranhão, Brazil.

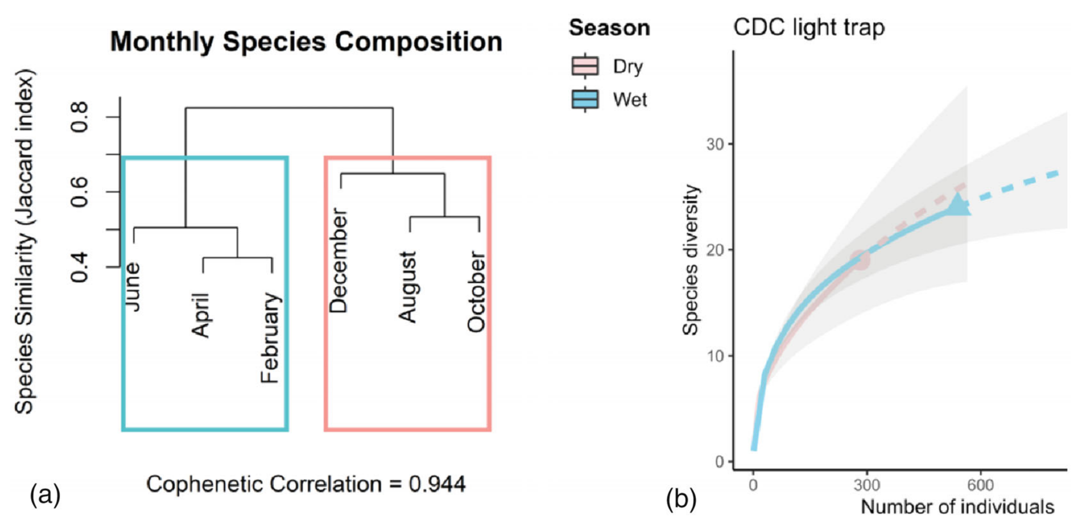

FIGURE 6 (a) Cladogram based on Jaccard's similarity index of sand fly species in the dry (August–December 2022) and wet (February–June 2023) seasons in the Santana IV rural settlement, Codó, Maranhão State, Brazil, and (b) rarefaction curves representing the cumulative richness of sand fly species captured on CDC traps.

marked decrease in temperatures during winter. As a result, the frequency of individuals reduces markedly (Pinheiro et al., 2016; Pinheiro et al., 2021; Rangel & Lainson, 2009; Tachinardi, 2012).

In the current study, most species were constant. The predominance of constant and accessory species over accidental ones is explained by the environmental characteristics of the surveyed area. For instance, the study area is anthropized and has degraded vegetation, among other aspects (Penha et al., 2013). The irregular distribution of accidental and accessory species is attributed to the different responses to blood meal sources, breeding sites and shelters near traps (Martin & Rebêlo, 2006).

Generally, sand flies are found in greater abundance in peridomestic environments, where domestic animals serve as blood meal sources for females and animal shelters serve as concentration points for males seeking to copulate with females (Andrade et al., 2014; Martin & Rebêlo, 2006; Oca-Aguilar et al., 2024; Ribeiro da Silva et al., 2019; Sales et al., 2019). However, in this study, sand flies were captured at a 1:1 male/female ratio in peri- and extradomestic environments. It is likely that the large quantities of *Ps. wellcomei* captured in April influenced their high abundance in the extradomestic environment.

Black Shannon traps captured nearly three times more sand flies than white traps. However, the sample obtained with white traps exhibited the highest species richness. For example, *Br. brumpti*, *Ev. termitophila*, *Lu. gomezi*, and *Mi. trinidadensis* were captured with white traps. The attraction of some sand fly species to white Shannon traps can be attributed to a combination of environmental factors, behavioural factors and light sensitivity. The mechanisms underlying this preference require further investigation, including behavioural studies and analyses of the spectral sensitivity of different species. This information is essential for the development of effective monitoring and control strategies aimed at these disease vectors (Briscoe & Chittka, 2001; Lima-Neto et al., 2018; Zorrilla et al., 2021).

The greater abundance of specimens in black Shannon traps corroborates the results of Hesam-Mohammadi et al. (2014). The authors captured more specimens using this type of trap. Other studies have shown that certain anthropophilic sand fly species are more attracted to black traps, such as *Lu. almerioi* Galati & Nunes 1999, *Lu. dispar* Martins & Silva, 1963, and *Pintomyia fischeri* (Pinto, 1926) (Galati et al., 2001; Infran et al., 2017). By contrast, *Migonemyia migonei* (França, 1920) prefers white traps (Moschin et al., 2013). Interestingly, *Ny. neivai* (Pinto, 1926) and *Ny. intermedia* were found to be attracted to both colours, but the first species was most attracted to white traps and the second was most attracted to black traps (Galati, Marassá, Fonseca, et al., 2010; Galati, Marassá, Gonçalves-Andrade, et al., 2010).

Here, the vector *Ps. wellcomei* was captured in both black and white Shannon traps, with a predominance of female specimens (1:3). These traps generally attract more anthropophilic species than other types of traps, explaining our findings, as only females feed on the blood of vertebrates (Brilhante et al., 2017). This result is in agreement with those of Castellón et al. (1994, 2000), Cabanillas et al. (2001) and Souza et al. (2016), who used white Shannon traps and human bait in the Brazilian and Peruvian Amazon. The authors found a high density of females of *Psychodopygus*.

The higher performance of Shannon traps compared with CDC traps was also reported by Casagrande (2018). The referred authors argued that the best method of sand fly capture depends on the researcher's objectives. For analysis of sand fly diversity, the best choice is CDC-type light traps, which attract several species. When the goal is to capture large quantities of anthropophilic sand flies for detection of *Leishmania*, it is best to use Shannon-type traps.

Two species stand out from an epidemiological point of view. *Psychodopygus wellcomei* was the most abundant species and has a well-known role in the transmission of *L.* (*V.*) *braziliensis*, the etiological agent of mucocutaneous leishmaniasis (Ready et al., 1983). This sand fly was mostly found in forest areas. *Nyssomyia whitmani*, an eclectic species, occurs in forest fragments, peri-urban areas, and peridomestic rural environments and feeds on animal and human blood (Carvalho-Silva et al., 2022; Guimaraes-e-Silva et al., 2017; Leonardo & Rebêlo, 2004; Ribeiro da Silva et al., 2019; Silva et al., 2015). The abundant presence of this species in peridomestic environments represents an increased risk of CL transmission to humans and domestic animals, given that these individuals feed on the blood of both humans and animals (Salomón et al., 2022; WHO, 2023).

In this study, *Lu. longipalpis* was considered an accessory species (CI = 33.3%). Only two individuals of the species were captured, demonstrating that the study locality still maintains a certain degree of vegetation preservation. The vector is highly adapted to urban environments with degraded vegetation (Caraballo & Arrivillaga, 2010). In the municipality of Codó, *Lu. longipalpis* holds greater importance in endemic areas of VL, where large populations of the sand fly are observed (Silva et al., 2015).

Overall, the findings of this study are relevant for health management agencies, contributing to decision-making in the control of adult sand flies during reproduction periods. The information can guide educational actions aimed at increasing the awareness of residents and preventing the transmission of leishmaniasis (OPS, 2023).

CONCLUSION

The findings demonstrate that the Santana IV settlement has undergone changes in land use and cover with the growth of human populations over the years. With this, the settlement has expanded and lands have been used for subsistence agriculture. The sand fly assemblage in the area is highly diverse, with 20 identified species. *Psychodopygus wellcomei* and *Ny. whitmani*, putative vectors of *Leishmania* spp., were the most abundant species. Changes in land use caused by human activities create new habitats for sand flies, altering the chain of transmission of leishmaniasis. These data are valuable for developing surveillance strategies, implementing control measures and promoting environmental and health education in collaboration with the community to reduce the risk of CL.

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AUTHOR CONTRIBUTIONS

Maxcilene da Silva de Oliveira: Conceptualization; investigation; methodology; visualization; writing – original draft; writing – review and editing; data curation; software; formal analysis. **Rosa Cristina Ribeiro da Silva:** Data curation; conceptualization; formal analysis; investigation; methodology; writing – review and editing; visualization. **Antonia Suely Guimarães e Silva:** Conceptualization; investigation; methodology; data curation; writing – review and editing; supervision; visualization. **Judson Chaves Rodrigues:** Investigation; methodology; data curation; visualization. **Ronayce Conceição de Jesus Serrão Pimenta:** Investigation; methodology; data curation; visualization. **Francisco Santos Leonardo:** Investigation; methodology; visualization. **José Manuel Macário Rebêlo:** Conceptualization; visualization; writing – review and editing; writing – original draft. **Valéria Cristina Soares Pinheiro:** Conceptualization; data curation; supervision; visualization; project administration; writing – review and editing; writing – original draft; funding acquisition.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw data are available from the corresponding author upon request. Data sharing is available in DOI: https://doi.org/10.5061/dryad.jwstqjqm5.

ETHICS STATEMENT

All collections were carried out under a Permanent Licence for Collection of Zoological Material issued by the Authorization and Information System in Biodiversity (SISBIO, process No. 11965-2).

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