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Assessing the importance of four sandfly species (Diptera: Psychodidae) as vectors of *Leishmania mexicana* in Campeche, Mexico

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Abstract. Localized cutaneous leishmaniasis represents a public health problem in many areas of Mexico, especially in the Yucatan Peninsula. An understanding of vector ecology and bionomics is of great importance in evaluations of the transmission dynamics of *Leishmania* parasites. A field study was conducted in the county of Calakmul, state of Campeche, during the period from November 2006 to March 2007. Phlebotomine sandfly vectors were sampled using Centers for Disease Control light traps, baited Disney traps and Shannon traps. A total of 3374 specimens were captured in the two villages of Once de Mayo (93.8%) and Arroyo Negro (6.1%). In Once de Mayo, the most abundant species were *Psathyromyia shannoni, Lutzomyia cruciata, Bichromomyia olmeca olmeca* and *Psychodopygus panamensis* (all: Diptera: Psychodidae). The Shannon trap was by far the most efficient method of collection. The infection rate, as determined by *Leishmania mexicana*-specific polymerase chain reaction, was 0.3% in Once de Mayo and infected sandflies included *Psy. panamensis*, *B. o. olmeca* and *Psa. shannoni*. There were significant differences in human biting rates across sandfly species and month of sampling. Ecological niche modelling analyses showed an overall overlap of 39.1% for the four species in the whole state of Campeche. In addition, the finding of nine vector–reservoir pairs indicates a potential interaction. The roles of the various sandfly vectors in Calakmul are discussed.

Key words. *Leishmania mexicana*, ecological niche, Mexico, Phlebotomine sandflies.

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Introduction

In Mexico, leishmaniasis represents a serious health problem. Although the disease is largely considered to be neglected, it is currently recognized that 25 of 32 states in Mexico have at least one reported case of leishmaniasis (Sánchez-Tejeda *et al.*, 2001; SSA 2014). Four clinical forms of leishmaniasis are known: localized cutaneous leishmaniasis (LCL); diffuse cutaneous leishmaniasis (DCL); mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). However, LCL represents by far the most prevalent clinical manifestation (Maroli *et al.*, 2013) and it is estimated that around 16 million people in Mexico live in areas in which they are at risk for the transmission of this form of the disease (Ramsey *et al.*, 2013). Although the disease is likely to have been present in Mexico since pre-Colombian times, it was first described in 1912 among chicle-gum collectors working in the forest of the Yucatan Peninsula (YP) under the designation 'Chiclero´s ulcer' (Seidelin, 1912). In the YP, the causative agent of the cutaneous form has been identified mainly as *Leishmania* (*Leishmania*) *mexicana* (Biagi) (Kinetoplastida: Trypanosomatidae), although, on a few occasions, the parasite *Leishmania* (*Viannia*) *braziliensis*, has also been reported (Canto-Lara *et al.*, 1999; Hernández-Rivera *et al.*, 2015). Official data of the Unique Information System for Epidemiological Surveillance, generated by the Health Secretariat in Mexico, indicate that 2322 cases of cutaneous leishmaniasis occurred in Campeche in the period 1990–2014, and represent 12.57% of all cases recorded in Mexico (SSA 2014). In addition, a recent paper published by Hernández-Rivera *et al.* (2015) provides an update of cases in some villages of Calakmul. Actual incidence and prevalence rates are basically unknown or underestimated because leishmaniases are largely unreported to the Health Secretariat as they are either undiagnosed or are not considered communicable diseases. The only rough estimate comes from the small-scale study conducted by Andrade-Narváez *et al.* (1990), who reported an incidence rate of 5.08 per 1000 inhabitants in Campeche. It should be pointed out, however, that incidence and prevalence rates may vary geographically and temporally. In addition, Andrade-Narváez *et al.* (1990) found that, at least in Campeche, the most exposed population and hence the highest-risk group were males aged 14–45 years, who are usually involved in various forms of employment within the forest.

In the YP, *L. mexicana* is maintained in the wild in enzootic cycles among several species of small mammals such as *Ototylomys phyllotis* (Merriam), *Peromyscus yucatanicus* (J. A. Allen & Chapman), *Sigmodum hispidus* (Say & Ord) and *Oryzomys melanotis* (Thomas) (Chablé-Santos *et al.*, 1995; Van Wynsberghe *et al.*, 2000, 2009). However, these findings do not exclude the possibility that *L. mexicana* circulates among other hosts because it has been found in other mammals (e.g. bats) in Mexico (Berzunza-Cruz *et al.*, 2015).

It is well known that phlebotomine sandflies are the vectors of *Leishmania* sp. worldwide (Killick-Kendrick, 1999). However, the full incrimination of a given sandfly species as a vector of *Leishmania* is extremely hard to demonstrate (Pozio *et al.*, 1985). Currently, there is increasing evidence that several species of sandfly harbour infection with several species of *Leishmania*, even in the same geographical region (Ready, 2013). Of approximately more than 800 species of sandfly worldwide, only 98 are so far known to be involved in the transmission of *Leishmania* (Maroli *et al.*, 2013). In Mexico, 48 species of sandfly have been collected and identified (Young & Duncan, 1994; Rebollar-Téllez *et al.*, 1996a, 2004, 2005; Ibáñez-Bernal *et al.*, 2004, 2006, 2010, 2015; Ibáñez-Bernal, 2005a, 2005b). The state of Campeche is rich in sandfly diversity: 22 species of sandfly have been identified, representing 45.8% of known species in the country (Rebollar-Téllez *et al.*, 1996a, 2004, 2005; Ibáñez-Bernal, 2005a, 2005b; Pech-May *et al.*, 2010).

The first sandfly species to be fully incriminated as a vector of *L. mexicana* was *Bichromomyia olmeca olmeca* (= *Lutzomyia olmeca olmeca*) (Vargas & Díaz-Nájera), according to classic studies carried out in Cariilo Puerto, Quintana Roo by Dr Francisco Biagi and his group (Biagi *et al.*, 1965). In this study, naturally infected flies were found and female *B. o. olmeca* (referred to as *Phlebotomus flaviscutellatus*) were shown to be capable of transmitting *Leishmania* parasites to hamsters and human volunteers (Biagi *et al.*, 1965). Other studies in the YP (Cruz-Ruiz *et al.*, 1994; Rebollar-Téllez *et al.*, 1996a, 1996b, 1996c, 2005; Canto-Lara *et al.*, 2007; Pech-May *et al.*, 2010; Sánchez-García *et al.*, 2010) provided evidence on the importance of other sandfly species in the transmission of *L. mexicana*. Specifically in Campeche, Pech-May *et al.* (2010) performed polymerase chain reaction (PCR) to detect infection, and measured sandfly abundances and human biting rates for several species of sandfly, including *Lutzomyia cruciata* (Coquillet), *Psychodopygus panamensis* (= *Lutzomyia panamensis*) (Shannon) and *Psathyromyia shannoni* (= *Lutzomyia shannoni*) (Dyar), and proposed that these species may be strongly suspected of being vectors.

Distributions and shifts in the ecological niches of sandfly species have been associated with a number of biotic and abiotic factors, of which those related to rainfall, temperature, altitude, latitude and physical barriers, as well as the abundance and distribution of vertebrate hosts, are of particular importance (Stephens *et al.*, 2009; Moo-Llanes *et al.*, 2013). These factors affect the temporal and spatial distributions of sandfly vectors, and thereby also alter the epidemiology and dynamics of disease transmission (Moo-Llanes *et al.*, 2013). The few studies that have been conducted in Mexico regarding the modelling of ecological niches of sandfly vectors have found that four species have overlapping ranges of distribution in areas of recurrent transmission of leishmaniases: the distributions of *B. o. olmeca* and *Psy. panamensis* are restricted to areas in southeastern Mexico, whereas other species, such as *L. cruciata* and *Psa. shannoni*, are widely distributed (González *et al.*, 2011). Furthermore, Moo-Llanes *et al.* (2013) predicted that, under the current scenario of climate change, ecological niche distributions of several species of phlebotomine sandfly of medical importance will increase substantially by the year 2080. The consequence of this expansion in sandfly distribution will be enhanced exposure of the human population to vectors capable of transmitting disease.

Therefore, studies on sandfly bionomics are crucial and must refer to seasonality, abundances and infection rates because, in combination, these provide strong evidence on the relative vectorial role of each sandfly species (Pech-May *et al.*, 2010). In addition, studies on the ecology of sandfly vectors are equally important because they are helpful for understanding the processes involved in the complex dynamics of transmission of *L. mexicana*. In the present study, patterns of abundance, as well as human biting and infection rates of sandfly species were investigated in two villages in Campeche which had not been studied previously despite reported cases of leishmaniases. The study also aimed to document the species composition of sandfly communities in the two villages and, using that information, to model the pattern of ecological niche distribution of sandfly species in relation to that of reservoir hosts.

Materials and methods

Study area

Sampling was carried out in the county of Calakmul, state of Campeche, in two selected villages: Once de Mayo and Arroyo Negro (Fig. 1). Unpublished reports gathered by the health authorities in the county of Calakmul, in addition to the cases reported by Hernández-Rivera *et al.* (2015), indicate that both villages have registered cases of leishmaniasis in previous years. According to the general classification of vegetation, both sites are located within the area designated as medium-high subperennial tropical forest (Flores & Espejel, 1994). The mean annual temperature is 22 °C and annual rainfall lies in the range of 1438–1561 mm, which classifies the climate as type AW_2 (summer rains) according to García (1996). The main economic activities of the inhabitants of both villages include agriculture and, to a lesser degree, livestock farming, bee-keeping and hunting.

Collection and identification of sandflies

Field collections were conducted during the months of November 2006, and January and March 2007. This period was chosen because it had previously been established that transmission of *L. mexicana* in Campeche occurs during those months (Andrade-Narváez *et al.*, 2003). Collections of sandflies were carried out over three consecutive nights during each month in each village. Sampling was carried out using five Centers for Disease Control (CDC) light traps per night (Sudia & Chamberlain, 1962), five Disney traps per night (Disney, 1968), baited with *Mus musculus* (Linnaeus), and two Shannon traps per night (Shannon, 1939). At each village, the footpaths normally used by villagers when entering the forest around the settlements were evident. These footpaths were used to select a patch of forest and to establish a linear transect. Disney traps and CDC light traps were set at every 15–20 m and each type of trap was randomly selected for each sampling night to minimize bias. All traps were set at dusk (18.00 hours) and were retrieved at dawn (07.00 hours approximately). The Shannon traps were set at a distance of 500 m from the other traps and were operated by two protected human volunteers from 18.00 to 22.00 hours. This time period was selected based on previous studies in which activity of sandflies had been reported (Biagi *et al.*, 1966; Rebollar-Téllez *et al.*, 1996c).

Sandflies were sorted during the early morning hours and preserved in 8-mL vials containing 70% ethanol. Female flies were separated for PCR amplification analysis and stored individually in 200-μL DNA/RNA-free Eppendorf vials containing 20 μL of analytic grade ethanol. Flies caught in Disney traps were submitted to an extra step. In order to remove excess oil from specimens collected using Disney traps, sandflies were washed with a mixture of 2% liquid detergent (Extran®; MA02, pH 7.2; EMD Chemicals, Inc., Gibbstown, NJ, U.S.A) and distilled water. All vials were labelled according to site, date, trap type, time and other information regarding collectors. Flies that had been kept in 8-mL vials were processed for identification purposes following the method of Ibáñez-Bernal (2005a, 2005b). Species identification was carried out using the keys of Young & Duncan (1994) and Ibáñez-Bernal (2000, 2005a, 2005b) by examining taxonomically important features under a microscope (Axiostar plus 176045; Carl Zeiss Meditec, Inc., Dublin, CA, U.S.A.). The classification proposed by Galati (1995, 2003) and the genera abbreviation system proposed by Brisola-Marcondes (2007) were adopted. For species identification using PCR analysis, the head and abdominal segments VI–IX of each individual were dissected. Voucher specimens were held in the entomological collection of Laboratorio de Entomología Médica de la Facultad de Ciencias Biológicas de la Universidad Autónoma de Nuevo León (UANL; Laboratory for Medical Entomology, Faculty of Biological Sciences, Autonomous University of Nuevo León).

DNA extraction and amplification of Leishmania *sp.*

Genomic Dexoribonucleic Acid (gDNA) extraction was performed for each sandfly using the phenol–chloroform protocol (Sambrook *et al.*, 1989), with modifications by Pech-May *et al.* (2013). To amplify DNA of *Leishmania*, a pair of primers based on kinetoplast minicircles were used, which amplify approximately 700 bp (Kato *et al.*, 2005): 5′-CTR GGG GTT GGT GTA AAA TAG-3′ (L.MC-1S), and 5′-TWT GAA CGG GRT TTC TG-3′ (L.MC-1R). The total volume of the PCR mix was 25 μL and included 12.5 μL of master mix (GoTaq Green Master Mix, 2X; Promega Corp., Madison, WI, U.S.A.), 100 ng of each primer and 50 ng of DNA template. Amplification took place in an iCycler thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.) using a programme of 95 °C for 2 min, followed by 30 cycles of 1 min at 95 °C, 1 min at 55 °C and 1 min at 72 °C, followed by 10 min at 72 °C and a final holding step at 4 °C. In samples that indicated positivity to *Leishmania*, a second PCR was carried out to identify species. Amplification of *L. mexicana* was performed using the primers described by Cupolillo *et al.* (1995) (IR1 5′-GCT GTA GGT GAA CCT GCA GCA GCT GGA TCA TT-3′) and Berzunza-Cruz *et al.* (2009) (LM17 5′-CCC CTC TCC TCC TCC CC-3′), which amplify approximately 835 bp. The final volume of the PCR reaction was 50 μL and included 25 μL of master mix (GoTaq Green Master Mix, 2X; Promega Corp.), 100 ng of each primer and 50 ng of DNA template. Amplification was performed in an iCycler thermocycler (Bio-Rad Laboratories, Inc.) as follows: an initial step at 5 min at 94 °C, followed by 35 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 1 min, and a final extension of 72 °C for 7 min followed by 4 °C indefinitely. The PCR products were visualized in agarose gel at 2%, stained with ethidium bromide

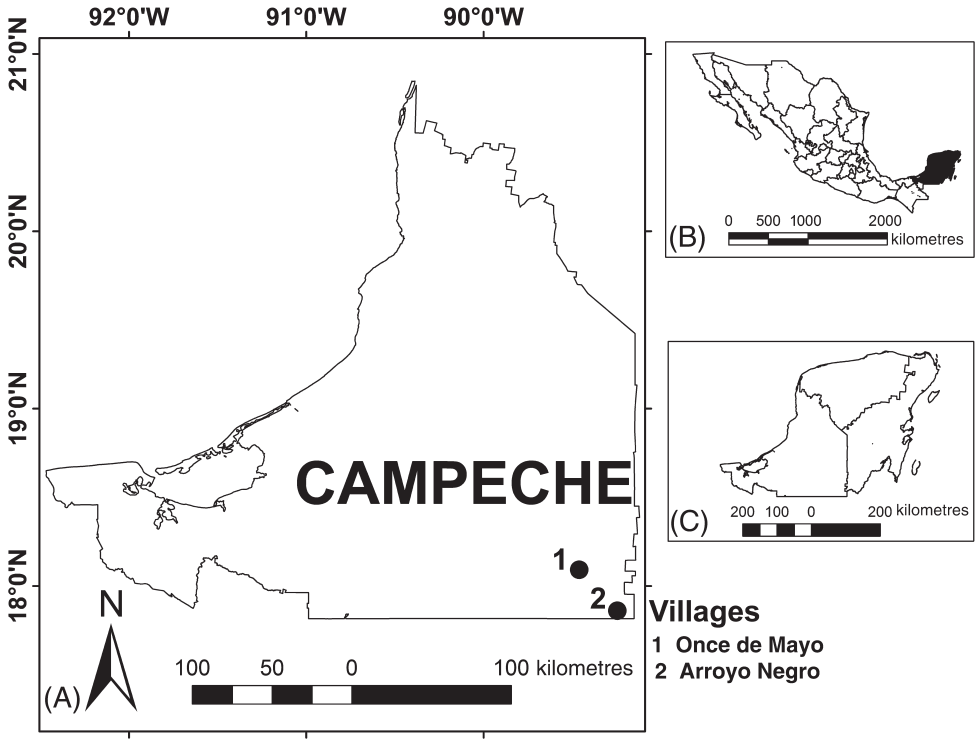

Fig. 1. The study sites in Campeche in the context of (A) the state of Campeche, (B) all of Mexico, and (C) the Yucatan Peninsula, which comprises the states of Yucatan, Campeche and Quintana Roo. Sandflies were collected during November 2006, and January and March 2007.

(0.5 μg/mL) under ultraviolet light (330 nm). A photographic record of gels was maintained using a photo documentation system (EDAS 290 Version 3.5.4; Eastman Kodak Co., Rochester, NY, U.S.A.).

Ecological niche model

A database was constructed from records previously collected in Campeche as reported in Moo-Llanes *et al.* (2013) for *B. o. olmeca* ($n = 10$ datapoints), *L. cruciata* ($n = 21$ datapoints), *Psy. panamensis* ($n = 19$ datapoints) and *Psa. shannoni* ($n = 21$ datapoints). Nine layers of bioclimatic data were considered: annual mean temperature; temperature seasonality; maximum temperature in the warmest month; minimum temperature in the coldest month; annual temperature range; annual precipitation; precipitation in the wettest month; precipitation in the driest quarter, and precipitation seasonality (Cuervo-Robayo *et al.*, 2013). Four layers of topographic data were considered: aspect; slope; topographic index, and elevation (EROSC 2014). The ecological niche model (ENM) was based on occurrence data, and bioclimatic and topographic layers were constructed using the algorithm GARP (genetic algorithm for rule-set prediction) (Stockwell & Peters, 1999). For GARP, a convergence criterion of 0.01 and a maximum of 1000 iterations were used, and a consensus of replicate model was achieved via a 20% relative omission threshold, retaining the central 50% of the distribution of proportional areas predicted as suitable. The software randomly divides occurrence points into training data for model building (75%) and test data for model testing (25%) (Anderson *et al.*, 2003). For the niche identity test, ENMs were generated for each vector and previously confirmed reservoirs of *Leishmania* sp. in Campeche: *Or. melanotis* ($n = 19$ datapoints); *Ot. phyllotis* ($n = 83$ datapoints); *P. yucatanicus* ($n = 68$ datapoints), and *S. hispidus* ($n = 60$ datapoints). Occurrences of species were downloaded in the Global Biodiversity Information Facility (GBIF 2014). Later, a maximum entropy-based algorithm, MaxEnt (Phillips *et al.*, 2006), was used for identity testing using ENMtools Version 13.3 (Warren *et al.*, 2010). The parameters used to confirm identity were: random test percentage (75%); replicated run type (bootstrap); maximum iterations (500), and threshold rule (minimum training presence) (Moo-Llanes *et al.*, 2013).

Data analyses

Data analyses were performed on three traits: (a) infection rate; (b) human biting rate, and (c) ecological niche characteristics. In the present study, only the four most abundant sandfly species, which were also of medical importance, were considered in the analysis. These were *B. o. olmeca*, *L. cruciata*, *Psy. panamensis* and *Psa. shannoni.* The infection rate was based on the proportion of infected sandfly species divided by the total number of flies examined using PCR. This proportion was incorporated into the mathematical model from Rabinovich & Feliciangeli (2004) modified by Pech-May *et al.* (2010) [(proportion of infected sandflies) (mean human biting rate ± standard error) (30 days in a month)] to estimate the number of potentially infectious bites per month. Both monthly and nightly human biting rates were calculated in relation to the total number of females collected in Shannon traps, taking into account the number of human volunteers and number of sampling nights. Data from Shannon traps were used as a proxy to estimate the mean (± standard error of the mean) human biting rate for each sandfly species/night (or month)/person. Biting estimates are a useful parameter with which to theoretically predict the number of infectious bites to which a given person living in the area would be exposed per month. Analysis of variance (ANOVA) was performed in Minitab Version 11.0 (Minitab, Inc., State College, PA, U.S.A.) and mean comparisons were carried out using the Tukey–Kramer method; all tests were considered significant if they achieved a *P*-value of <0.05 (Sokal & Rohlf, 1995). The shift in ecological niche distribution was calculated for each sandfly species. The proportion of pixels occupied was calculated by the total number of pixels in the whole state of Campeche. The total percentage of overlap among the distributions of all four species of sandfly was calculated for the whole state. To extrapolate the total population at risk, 2010 census data from the National Institute of Geography and Statistics of Mexico (INEGI) (INEGI 2014) were used. Outcomes were later recalculated for rural localities (<10 000 inhabitants) and urban localities (>10 000 inhabitants). 'Hellinger's distances' for all pairwise combinations of vector–rodent species (*Or. melanotis*, *Ot. phyllotis*, *P. yucatanicus*, *S. hispidus*) were calculated. The hypothesis that species occupy identical niches was rejected if the empirical observed value for Hellinger's distance was significantly lower than the value expected from the pseudoreplicated datasets (Warren *et al.*, 2010).

Results

Species composition by capture method

A total of 3374 phlebotomine sandflies were collected in the two villages in Campeche. Most of the specimens (93.8%) were caught in Once de Mayo and belonged to nine species. The most abundant were *Psa. shannoni* (47.8%), *L. cruciata* (18.2%), *B. o. olmeca* (18.1%), and *Psy. panamensis* (7.1%); the remaining five species together represented 8.6% of the sandflies collected. Shannon traps collected 91.4% of all sandflies caught; the most abundant species caught in these traps was *Psa. shannoni* (51.9%). CDC light traps collected a smaller fraction of sandflies (3.6%), among which the most abundant species was *B. o. olmeca* (50.8%). Disney traps accounted for a 4.9% share of the total collection, in which *B. o. olmeca* (90.3%) was the most abundant species (Table 1). In Arroyo Negro, 206 specimens (6.1% of all sandflies caught) belonging to nine species were collected. These included *L. cruciata* (40.7%), *B. o. olmeca* (17.4%), *Psa. shannoni* (14.0%) and *Psy. panamensis* (9.7%); the other five species together represented 17.1% of the local collection. As in Once de Mayo, the majority (74.2%) of specimens collected in Arroyo Negro were caught in Shannon traps, but the most abundant species was *L. cruciata* (representing 48.3% of all sandflies collected using Disney traps). Captures using CDC traps placed in Arroyo Negro represented 20.8% of collections and *Dampfomyia deleoni* (Fairchild & Hertig) (Diptera: Psychodidae) was the most abundant (46.5%) species caught. Finally, Disney trap collections represented 4.8% of collections made in Arroyo Negro and *B. o. olmeca* was the most abundant sandfly species (70.0%) collected (Table 1). Comparisons of the abundances of the four species of suspected medical importance (*B. o. olmeca*, *L. cruciata*, *Psy. panamensis* and *Psa. shannoni*) showed significant differences in the number of flies caught between months ($F = 4.3$; d.f. = 3,15; $P = 0.02$) in Once de Mayo, but not in Arroyo Negro ($F = 1.3$; d.f. = 3,15; $P = 0.29$).

Sandflies naturally infected by L. mexicana

A total of 1492 female sandflies were analysed by PCR to detect possible *Leishmania* infection (1371 from Once de Mayo and 121 from Arroyo Negro). The proportion of infection in Once de Mayo was 0.3% and all of the three *Leishmania*-positive sandflies infected, *Psy. panamensis* (one of 82, 1.2%), *B. o. olmeca* (one of 342, 0.3%) and *Psa. shannoni* (one of 412, 0.2%), were infected with *L. mexicana*. Two of the three positive samples were obtained in Shannon traps and included one *Psy. panamensis* caught in November (18.00–18.59 hours) and one *Psa. shannoni* caught in March (20.00–20.59 hours). The remaining infected sandfly, *B. o. olmeca*, was caught in January with a Disney trap. Another positive sandfly was obtained in March, but its species could not be identified. No *L. mexicana*-infected sandflies were collected in Arroyo Negro.

Human biting activity

Human biting activity was estimated for the medically important species *B. o. olmeca*, *L. cruciata*, *Psy. panamensis* and *Psa. shannoni*. In Once de Mayo, the mean number of *B. o. olmeca* bites varied significantly across the months of collection ($F = 7.7$; d.f. = 3,15; $P = 0.01$). Biting activity in *L. cruciata* was highest in November and December, whereas biting activity in *B. o. olmeca* peaked in January, when it was higher than rates in the other three species. *Psathyromyia shannoni* exhibited the highest activity in March (Fig. 2A). In Arroyo Negro, mean numbers of sandflies caught in Shannon traps did not differ significantly across the months of study. The month in which the human biting rate of *Psa. shannoni* was highest was November, whereas that for *L. cruciata* was December. *Bichromomyia o. olmeca* had the highest biting rates in January and *L. cruciata* displayed the highest biting rate in March (Fig. 2B). Mean human biting rates per hour differed significantly for all species studied in Once de Mayo ($F = 6.7$; d.f. = 3,15; $P = 0.00$) and in Arroyo Negro ($F = 7.2$; d.f. = 3,15; $P = 0.00$). Biting activity in all species was highest during the first study hour in both villages (18.00–18.59 hours) (Fig. 2C and D). The mean biting rate of the sandfly species was used to calculate the mean number of potentially infectious bites a given person would receive in a particular month (potentially infectious bites/person/month). Overall inoculation rates of *L. mexicana* in Once de Mayo were 0.3%

Table 1. Species composition and relative abundances of sandflies (Diptera: Psychodidae) caught at Once de Mayo (OM) and Arroyo Negro (AN), Campeche, Mexico, with three types of trap during November 2006, and January and March 2007.

	Sandflies, *n*											
	Shannon trap				CDC trap				Disney trap			
	OM		AN		OM		AN		OM		AN	
Sandfly species	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Bichromomyia olmeca olmeca (Vargas & Díaz-Nájera)	373	2	27	0	54	5	2	0	141	0	7	0
Brumptomyia galindoi (Fairchild & Hertig)	0	0	0	0	0	0	0	1	0	0	0	0
Dampfomyia deleoni (Fairchild & Hertig)	10	1	5	0	30	1	17	3	2	1	1	0
Lutzomyia cruciata (Coquillett)	563	2	73	1	9	0	9	0	3	0	1	0
Lutzomyia longipalpis (Lutz & Neiva)	2	0	1	0	0	0	0	0	0	0	0	0
Psathyromyia carpenteri (Fairchild & Hertig)	0	2	0	0	1	5	3	2	0	0	0	0
Psathyromyia shannoni (Dyar)	664	841	21	3	2	3	0	5	6	0	0	0
Psathyromyia undulata (Fairchild & Hertig)	185	0	0	0	0	0	0	0	0	0	0	0
Psychodopygus panamensis (Shannon)	167	51	18	0	5	1	1	0	2	1	1	0
Lutzomyia sp.	23	10	1	3	0	0	0	0	0	0	0	0
Absolute abundance	1987	909	146	7	101	15	32	11	154	2	10	0
Relative abundance by village	62.7%	28.6%	70.8%	3.3%	3.1%	0.4%	15.5%	5.3%	4.8%	0%	4.8%	0%
Average number of sandflies/trap/night	41.3	18.9	3.0	0.1	0.5	0.0	0.1	0.0	0.8	0.0	0.0	0.0

CDC, Centers for Disease Control.

in November and 0.2% in March: in Once de Mayo, mean biting rates of 4.2 ± 0.2 for *Psy. panamensis* and 5.9 ± 0.0 for *Psa. shannoni* were obtained for November and March, respectively.

Ecological niche model

The areas of distribution across Campeche amounted to 43.3% of the state for *B. o. olmeca* (Fig. 3A), 63.0% for *L. cruciata* (Fig. 3B), 63.1% for *Psy. panamensis* (Fig. 3C), and 64.9% for *Psa. shannoni* (Fig. 3D); the four species overlapped across 39.1% of the state. With the exception of El Carmen and Palizada, species overlaps occurred in all counties across the state. The total human population in Campeche is 815 126 (rural population: 328 009; urban population: 487 117). The human population exposed in rural and urban areas to different species of sandfly was highest for *Psa. shannoni* (rural: 172 086; urban: 51 448), followed by *Psy. panamensis* (rural: 152 930; urban: 66 382), *L. cruciata* (rural: 153 781; urban: 56 097) and *B. o. olmeca* (rural: 107 343; urban: none). Hellinger's distance allowed the identification of nine vector–rodent pairs, all of which shared similar ecological niches: *L. cruciata* and *Or. melanotis*; *L. cruciata* and *Ot. phyllotis*; *L. cruciata* and *S. hispidus*; *Psy. panamensis* and *Or. melanotis*; *Psy. panamensis* and *P. yucatanicus*; *Psa. shannoni* and *Or. melanotis*; *Psa. shannoni* and *P. yucatanicus*; *Psa. shannoni* and *Ot. phyllotis*, and *Psa. shannoni* and *S. hispidus* (Table 2).

Discussion

Cutaneous leishmaniasis has been considered endemic and an important health problem in the YP since Seidelin (1912) reported the first cases. Nowadays, in the YP, at least four sandfly species, *B. o. olmeca*, *L. cruciata*, *Psy. panamensis* and *Psa. shannoni*, are strongly suspected to be vectors of *L. mexicana* (Biagi *et al.*, 1965; Rebollar-Téllez *et al.*, 1996a, 1996b, 1996c, 2005; Canto-Lara *et al.*, 2007; Pech-May *et al.*, 2010; Sánchez-García *et al.*, 2010). In addition to their natural infection with *L. mexicana*, these species were shown to represent more than 80% of total sandfly populations in several villages of Calakmul and Escárcega, Campeche (Rebollar-Téllez *et al.*, 1996c, 2005; Pech-May *et al.*, 2010). Similarly, in the present study, the four species dominated collections performed in Once de Mayo (91.3%) and Arroyo Negro (82.0%). It is unclear why sandfly abundances in Once de Mayo ($n = 3168$) and Arroyo Negro ($n = 206$) were so proportionally different. One possible explanation is that the pattern of rainfall affected the activity of sandflies in Arroyo Negro. Although rainfall frequency and quantity were not measured and compared between the villages in the present study, qualitative observations indicated that more days of rain occurred in Arroyo Negro, which is the last human settlement in Mexico before the border with Guatemala and Belize. It is possible that the area is usually more humid because its vegetation has been better conserved. Climatic variables are known to modulate and/or influence sandfly populations, which are very sensitive to sudden changes in temperature and especially to changes in humidity or topography (Biagi & de Biagi, 1953; Chaniotis *et al.*, 1971; Morrison *et al.*, 1995; Rebollar-Téllez *et al.*, 1996a; Ostfeld *et al.*, 2004). One of the determining factors in the development of the immature sandfly is humidity and some species have been observed to present neotropical quiescence in the egg stage or in the pupal stage during periods of drought and heavy rain (Killick-Kendrick, 1999), affecting the productivity of adults. Thus, damp environments favour the hatching of larvae, yet excessive rain reduces the number of larvae (Lane, 1993). A study carried out in Venezuela showed that the abundance of *Lutzomyia spinicrassa* (Morales, Osorno-Mesa,

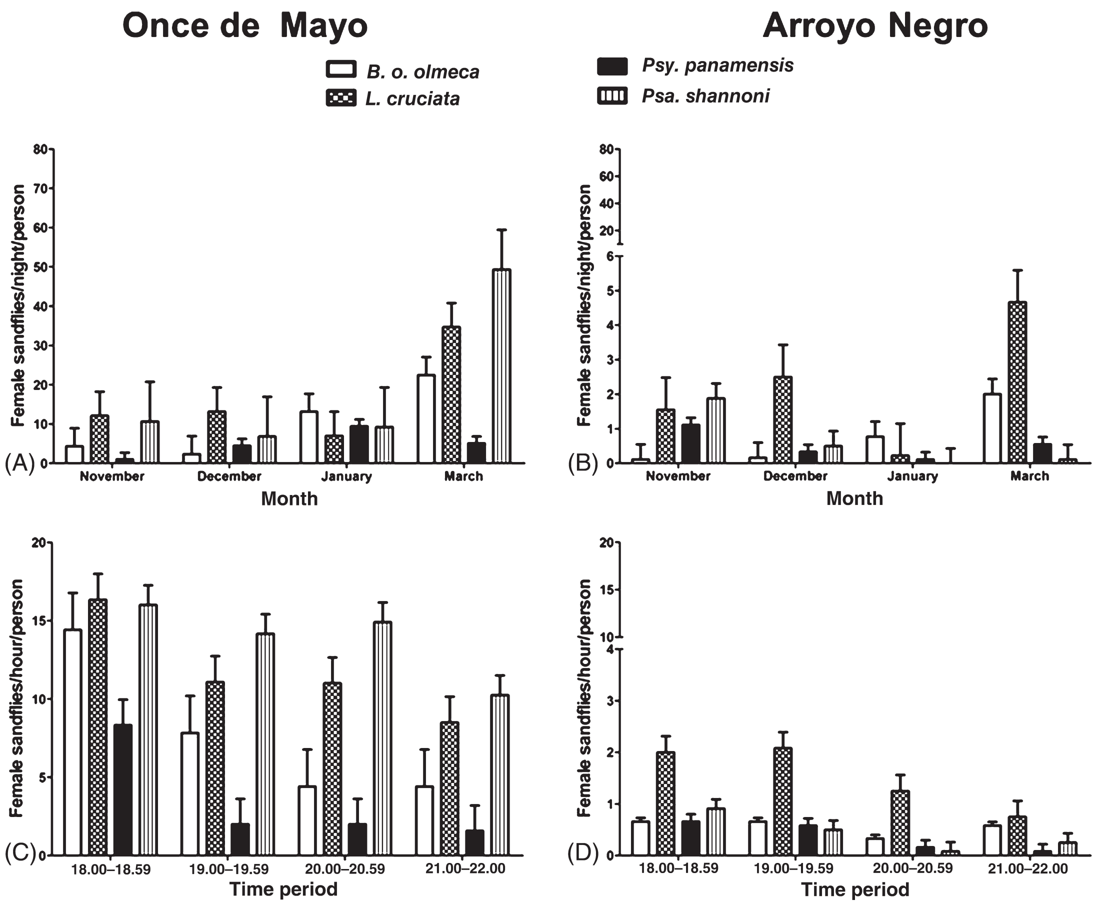

Fig. 2. Human biting rates per month and by night hours in four sandfly species caught in Shannon traps in the state of Campeche, Mexico. (A) Mean ± standard error (SE) monthly biting rate in Once de Mayo. (B) Mean ± SE monthly biting rate in Arroyo Negro. (C) Mean ± SE hourly biting rate in Once de Mayo. (D) Mean ± SE hourly biting rate in Arroyo Negro. *B. o. olmeca*, *Bichromomyia olmeca olmeca*; *L. cruciata*, *Lutzomyia cruciata*; *Psy. panamensis*, *Psychodopygus panamensis*; *Psa. shannoni*, *Psathyromyia shannoni.*

Osorno & Hoyos) was negatively correlated with precipitation (Perruolo *et al.*, 2006). Rebollar-Téllez *et al.* (1996a, 1996c) found that abundances of *L. cruciata* were almost null during the rainy months in the village of La Libertad, Campeche. Sánchez-García *et al.* (2010) showed that the biting rates of *B. o. olmeca*, *L. cruciata* and *Psa. shannoni* decreased during the rainy season in the state of Quintana Roo, Mexico. The current study presents new data on nine sandfly species found in two villages, which together represent approximately 40% of all sandfly species reported in the state of Campeche. At both study sites, the four most abundant sandfly species were those considered important in the transmission of leishmaniases to humans (*B. o. olmeca*, *L. cruciata*, *Psy. panamensis* and *Psa. shannoni*). These sandfly species have been previously reported as suspected vectors in Mexico (Biagi *et al.*, 1965; Cruz-Ruiz *et al.*, 1994; Rebollar-Téllez *et al.*, 1996a, 1996b, 1996c, 2005; Pech-May *et al.*, 2010; Sánchez-García *et al.*, 2010), Guatemala (Maroli *et al.*, 2013), Belize (Williams, 1966; Disney, 1968; Rowton *et al.*, 1991), Nicaragua (Zeledon & Murillo, 1983) and Costa Rica (Zeledón *et al.*, 1985).

Findings on relative abundances of sandfly species are influenced by the type of trapping method used (Alexander, 2000). In the present study, the two most common species caught with CDC light traps were *B. o. olmeca* (50.8%) and *D. deleoni* (46.5%) in Once de Mayo and Arroyo Negro, respectively. The sandfly *D. deleoni* has usually been captured in CDC light traps in previous studies in Mexico, in which its abundance has ranged from 37.6 to 72.1% (Rebollar-Téllez *et al.*, 1996c, 2005; Pech-May *et al.*, 2010). In rodent-baited Disney traps, *B. o. olmeca* was the most commonly caught species. This is in accordance with the findings of previous studies conducted in the YP (Rebollar-Téllez *et al.*, 2005; Pech-May *et al.*, 2010; Sánchez-García *et al.*, 2010).

In Once de Mayo, all medically important sandfly species were found to be most abundant in the month of March, with the exception of *Psy. panamensis*, which was most abundant in January. In Arroyo Negro, a similar pattern of peaks in abundance in March was observed in *B. o. olmeca* and *L. cruciata*, whereas *Psy. panamensis* and *Psa. shannoni* were most abundant in November. The present data are in accordance with earlier findings in the literature, in which the months of November to March are reported as those in which sandfly abundances peak, although monthly variations may occur at individual sites (Rebollar-Téllez *et al.*, 1996c; Pech-May *et al.*,

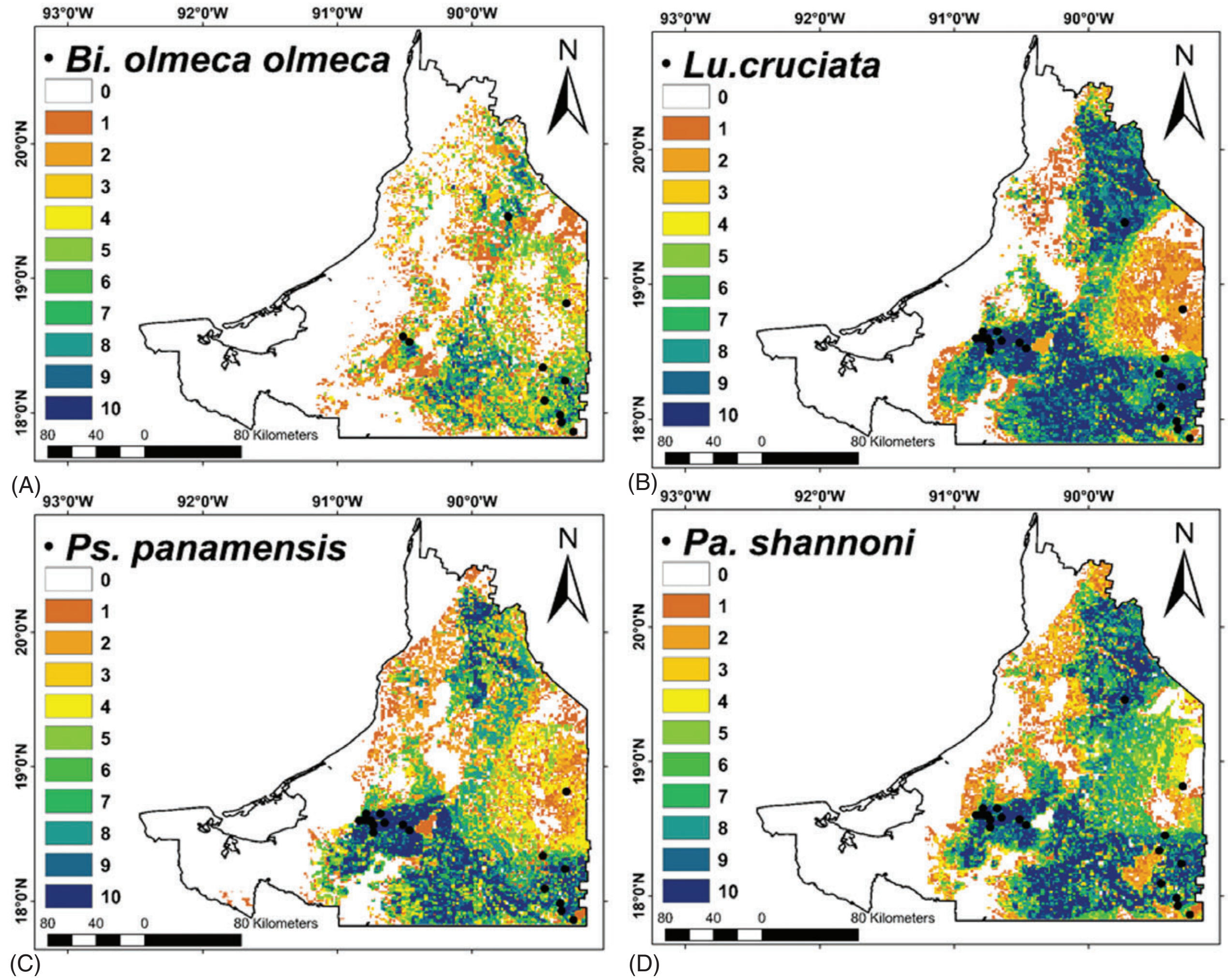

Fig. 3. Ecological niche distribution of four sandfly species of medical importance: (A) *Bichromomyia olmeca olmeca*; (B) *Lutzomyia cruciata*; (C) *Psychodopygus panamensis*, and (D) *Psathyromyia shannoni*. ENM, ecological niche model.

2010; Sánchez-García *et al.*, 2010). As with abundance data, it is likely that fluctuations in monthly biting rates will also occur. Biagi *et al.* (1965) reported that human bait collections were highest during 18.00–19.00 hours, a finding confirmed by other more recent studies (Rebollar-Téllez *et al.*, 1996c, 2005; Pech-May *et al.*, 2010; Sánchez-García *et al.*, 2010) and by the present observations.

The only village in which it was possible to detect *L. mexicana*-infected sandflies was Once de Mayo. However, the infection rate was very low (0.3%) compared with those found in other studies in the YP. For instance, Pech-May *et al.* (2010) found overall infection rates of 1.4 and 5.3% in Campeche in the villages of Dos Laguna Sur and 20 de Junio, respectively. In 20 de Junio, infection rates by species were 7.6% in *B. o. olmeca*, 0.8% in *L. cruciata*, 4.3% in *Psy. panamensis* and 15.1% in *Psa. shannoni* (15.1%). However, in a study carried out by Sánchez-García *et al.* (2010) in the state of Quintana Roo, the overall infection rate among sandfly species was shown to be 1.9% and infection rates by species were 1.5% in *B. o. olmeca*, 9.4% in *L. cruciata* and 8.0% in *Psa. shannoni*. Although the infection rate of sandfly species in Once de Mayo was low, it is interesting that it provides supportive evidence of the natural infection of the previously incriminated species *B. o. olmeca*, *Psy. panamensis* and *Psa. shannoni*. Furthermore, it confirms *B. o. olmeca* as the only *L. mexicana*-infected sandfly species to be caught in Disney traps. These findings agree with those of previous studies conducted in Quintana Roo (Sánchez-García *et al.*, 2010) and other locations in Campeche (Pech-May *et al.*, 2010).

The ENM is a recent implementation in Mexico for sandflies. The first study began with the work of González *et al.* (2010), who showed the potential distributions of six medically important sandfly species. Later, Moo-Llanes *et al.* (2013) further employed modelling tools on the larger scale of Central and North America with 28 species of sandfly. The distribution models obtained in the present study generated fine-scale information with which it was possible to detect focused distributions of sandflies. Using fine-scale data should make it possible

Table 2. Ecological niche similarity between sandfly species and reservoirs of *Leishmania* sp.

Sandfly species	Reservoir species	I_{obs}	I_{RET}
Bichromomyia olmeca olmeca	*Oryzomys melanotis*	0.85	0.42–0.87
	Ototylomys phyllotis	0.83	0.45–0.87
	Peromyscus yucatanicus	0.79	0.50–0.90
	Sigmodum hispidus	0.81	0.52–0.85
Lutzomyia cruciata	*Oryzomys melanotis*	0.90*	0.47–0.82
	Peromyscus yucatanicus	0.87	0.57–0.87
	Ototylomys phyllotis	0.94*	0.50–0.85
	Sigmodum hispidus	0.94*	0.45–0.85
Psychodopygus panamensis	*Oryzomys melanotis*	0.98*	0.42–0.80
	Peromyscus yucatanicus	0.94*	0.47–0.90
	Ototylomys phyllotis	0.96	0.85–0.96
	Sigmodum hispidus	0.99	0.85–0.99
Psathyromyia shannoni	*Oryzomys melanotis*	0.97*	0.47–0.82
	Peromyscus yucatanicus	0.95*	0.50–0.90
	Ototylomys phyllotis	0.98*	0.47–0.82
	Sigmodum hispidus	1.00*	0.52–0.85

*Significant value ($P < 0.05$).
I_{obs}, Hellinger's distance, I_{RET}, interval for random equivalence test.

to identify areas in which the risk for transmission is high. The four species of medical importance displayed an overlap of 39.1% across the whole state of Campeche. With the exception of El Carmen and Palizada, all counties in which these species have been reported exhibited a certain degree of overlap. However, these species of sandfly have not yet been reported in the counties of Champotón, Campeche, Tenabo and Hecelchakán, although these counties provide optimal conditions for their presence. González *et al.* (2010) described the ecological niches of *Lutzomyia diabolica* (Hall) as overlapping those of rodent hosts for *Leishmania* such as *Neotoma albigula*, *Neotoma floridana* and *Neotoma micropus*. More recently, ecological niche analysis carried out by Moo-Llanes *et al.* (2013) reported an overlap between the ecological niches of the sandfly vector *L. cruciata* with *Leishmania* sp. parasites in Mexico. The present results show that the sandfly *B. o. olmeca* is attracted mostly to rodent-baited traps. However, it is noteworthy that the ecological niche of this sandfly species does not exhibit any similarities with those of any of the four rodent host species for *L. mexicana*. This finding is surprising because the ecological niches of the other three sandfly species share similarities with those of the reservoir hosts for *L. mexicana*. Interestingly, *Psa. shannoni* was the only sandfly species to show a correlation in ecological niche with all four species of reservoir rodent considered in the YP (Chablé-Santos *et al.*, 1995; Van Wynsberghe *et al.*, 2000, 2009). At this stage it remains unknown why the niche of *B. o. olmeca* does not correlate with those of the rodents under study. One possible explanation is that *B. o. olmeca* is in fact more strongly associated with the niches of other species of rodent or other mammal hosts in the area. Another possibility is that biotic interactions occur on a smaller scale and could not be identified by the analyses performed herein. However, to confirm this possibility, more detailed studies on vector feeding patterns, host preferences and modelling of biotic network interactions are required.

In the YP, much effort has been put into understanding the epidemiology of leishmaniasis and determining the vectors that enable transmission of the pathogen. The present results support and further strengthen the evidence that four species, *B. o. olmeca*, *L. cruciata*, *Psy. panamensis* and *Psa. shannoni*, are the major vectors of *L. mexicana* in southern Mexico. In conclusion, the present data not only confirm previous suggestions regarding the vectorial roles of these sandfly species, but also provide novel evidence on the overlaps among the niches of sandfly vectors and those of rodent reservoir species.

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