

Original Article

Modeling Spatial Distribution of *Sergentomyia minuta* (Diptera: Psychodidae) and Its Potential Implication in Leishmaniasis Transmission in Morocco

Morocco Mohamed Daoudi¹; *Samia Boussaa^{1,2}; Ali Boumezzough¹

¹Laboratory of Ecology and Environment (L2E), (URAC 32), Cadi Ayyad University, Faculty of Sciences Semlalia, Marrakesh, Morocco

²ISPITS-Higher Institute of Nursing and Technical Health Occupations, Ministry of Health, Marrakesh, Morocco

(Received 07 Aug 2018; accepted 07 Mar 2020)

Abstract

Background: Leishmaniasis are parasitic diseases caused by *Leishmania* species and transmitted by the bite of sand flies. The genus *Lutzomyia* and *Phlebotomus* of sand flies are known to be the responsible vector for transmitting almost all *Leishmania* species to humans. The detection of *Leishmania* DNA in species of the genus *Sergentomyia*, in different regions, suggests their likely role in *Leishmania* transmission.

Methods: Our objective was to determine the potential geographical distribution of *Sergentomyia minuta*, the most dominant *Sergentomyia* species in Morocco, using ecological niche modeling.

Results: The results showed the widespread geographical distribution of *S. minuta* in Morocco, specifically in northern and central Morocco where visceral and cutaneous leishmaniasis foci occur. There were six abiotic factors affecting the distribution of *S. minuta* whose annual precipitation, precipitation seasonality and precipitation of driest month as the most important ecological variables of the model.

Conclusion: A positive statistical correlation between human leishmaniasis cases and *S. minuta* abundance was noted suggesting the potential involvement of *S. minuta* in local *Leishmania* transmission cycles.

Keywords: *Sergentomyia minuta*; Ecological niche modeling; Maximum entropy (MaxEnt); Morocco

Introduction

Phlebotomine sand flies (Diptera: Psychodidae) are small nocturnal insects of which only the female is a hematophagous. Currently, about 1000 species of sand flies are distributed in almost all biogeographic regions of the world (1). These insects play a crucial role in the epidemiology of relevant diseases, some being of great veterinary and medical importance. Indeed, certain species of sand flies are vectors of various infectious and parasitic agents such as agents of canine and human leishmaniasis, bartonellosis and several arboviruses. Allergic reactions can also be caused by the exposure of sand fly bites, though is not linked to the spread of disease (2).

Leishmaniasis remains the most related diseases to sand flies. The genera *Phlebotomus*, *Lutzomyia* and *Sergentomyia* contain all hematophagous species of sand flies (3), with only

the genus *Lutzomyia* in the New World, and *Phlebotomus* in the Old World responsible for transmitting almost all *Leishmania* species to humans (4, 5).

Like most countries around the Mediterranean, leishmaniasis in Morocco are a serious public health problem. These affections are widely represented, from the Moroccan Rif Mountains to the palm groves of the Moroccan Anti-Atlas (6). Three epidemiological entities are known in Morocco: zoonotic cutaneous leishmaniasis due to *Leishmania major*, anthroponotic cutaneous leishmaniasis due to *L. tropica* and visceral and cutaneous leishmaniasis due to *L. infantum* (6).

Regarding disease transmission, only vectorial role of species of the genus *Phlebotomus* (namely, *Phlebotomus papatasi*, *P. sergenti*, *P. ariasi*, *P. longicuspis* and *P. perniciosus*) was

investigated in Morocco (7–9); where sand fly fauna has 22 species including, nine species of the genus *Sergentomyia* (10): *Sergentomyia christophersi*, *S. clydei*, *S. africana*, *S. minuta*, *S. dreyfussi*, *S. schwetzi*, *S. antennata*, *S. fallax* and *S. lewisi*.

Considering the epidemiological status of leishmaniasis in Morocco, it is critically important to highlight the probable role as a vector for other sand fly species. Vector incrimination depends on the accumulation of evidence based mainly on its geographical and temporal abundance. According to many authors, *Sergentomyia* species are spread throughout Morocco, especially *S. minuta* and *S. fallax* (11, 12). *Sergentomyia minuta* is collected in urban as well as in rural area (13) up to 1,200m (14). It was considered as ubiquitous species in Morocco (15) with high density and a long activity period (16, 17).

In addition, *Leishmania* DNA was detected in species of the genus *Sergentomyia* in different regions suggesting their likely role in *Leishmania* transmission (18). Studies conducted in leishmaniasis foci in Iran, Mali and Portugal have reported the detection of *L. major* DNA in *Sergentomyia sintoni* (19), *S. darlingi* (20) and *S. minuta* (21). Earlier, it has also been isolated from *S. garnhami* in Kenya (22). Other reports also detected *L. donovani* DNA in *S. babu* in India (23) and more recently, *L. siamensis* detected in *S. gemmea* in Thailand (24). Moreover, several viruses have been isolated from sand flies of the genus *Sergentomyia* such as the *Saboya* virus, viruses RNA of ArD 95737 and ArD 111740 (25, 26), which has made it possible to suspect the vector role of *Sergentomyia* species (27).

Despite the presence and the abundance of *Sergentomyia* species in Morocco, no study has invested its potential involvement in leishmaniasis transmission cycles. Therefore, we used ecological niche modeling to identify the distribution of the most dominant species of *Sergentomyia* genus in Morocco and consequently discuss its potential involvement in local *Leish-*

mania transmission cycles.

Materials and Methods

Study area

Morocco is a northern African country, bordering the North Atlantic Ocean and the Mediterranean Sea. The current study covered sand fly sampling in 190 localities with altitude ranges between sea level and up to 2123m above sea (Fig. 1). These localities covered mainly northern and central Morocco, where human cutaneous and visceral leishmaniasis are endemic (28).

The climate in Morocco is mostly Mediterranean; however, seven bioclimatic regimes are occurred due to topographic differences across the country (Fig. 1). In 2014, the total human population in Morocco is about 33.7 million, with an urbanization rate of 60% (29).

Occurrence data and environmental variables

Occurrence records of sand flies were collected from our field survey (published and unpublished data) and literature using the keyword “species name, Morocco” as a search word to retrieve all data archived in PubMed database. Thus, about 70% of the 190 localities were investigated by the Ecology and Environment Laboratory (L2E) team between 2005 and 2016. The rest of the localities were published observations of the investigations in Morocco (20 publications on PUBMED). The coordinates of all localities were registered with a global positioning system (GPS).

For ecological niche modeling, 19 climatic variables (Bio1-Bio19) were used with elevation precision (ALT) for each locality (Table 1). Altitude and bioclimatic data were obtained from the WorldClim project (<http://www.worldclim.org>), with a spatial resolution of 30 arc seconds (about 1km²).

Construction of the model

The modeling was performed using Maximum entropy (MaxEnt) version 3.3.3 (30), which

uses an optimization procedure comparing the presence of the species with the characteristics of the environment, based on the principle of maximum entropy. This program allows for inference from incomplete information despite limited occurrence data (31–33).

Distribution data for the sand fly species and environmental data were imported into MaxEnt v. 3.3.3 (31), a total of 15 model replicates were run, with 30% of the points of presence used to test the model and 70% for model construction (34).

Model Evaluation

To evaluate the quality of model produced by MaxEnt, we analyzed the Operating Characteristic Curve Receiver Operating Characteristic (ROC) which assigns a unique value according to the model performance Area Under the Curve (AUC).

ROC analysis is a measure of sensitivity, which corresponds to the true positive rate (no error of omission), compared to the false positive rate (superfluous forecast error). Also, the ROC analysis evaluates the ability of the model to correctly predict the occurrence of the species. The closer the AUC value is to 1, the closer the correlation of the model (31) (Figs. 2, 3). The importance of the variables in explaining the potential geographic distribution of *S. minuta* was estimated using the Jackknife Environmental Variables Importance Test (31) (Table 2), which assesses the relative contribution (%) of the variables used to generate the distribution model produced by MaxEnt.

Statistical analysis

The Chi-squared test (χ^2) was used to analyze the correlation between variation of *S. minuta* abundance and the presence/absence of human leishmaniasis cases (cutaneous leishmaniasis by *L. tropica* and visceral leishmaniasis by *L. infantum*) in all sampling sites.

Epidemiological data (human cutaneous and visceral leishmaniasis cases) were obtained from official Moroccan Ministry of Health reports (28).

Results

In this study, we assembled 190 sampling sites for sand fly species. Five *Sergentomyia* species were noted: *Sergentomyia minuta* (N= 150), *S. fallax* (N= 110), *S. dreyfussi* (N= 57), *S. africana* (N= 10) and *S. christophersi* (N= 15). *Phlebotomus* species were also recorded, specifically *Phlebotomus sergenti* (N= 102), *P. papatasi* (N= 82), *P. longicuspis* (N= 78), *P. perniciosus* (N= 76) and *P. ariasi* (N= 43), while *P. alexandri* (N= 09), *P. kazeruni* (N= 03), *P. langeroni* (N= 02), and *P. mariae* (N= 02) were noted with very low reporting.

Sergentomyia minuta was the most predominant species and it was collected in the 150/190 sampling sites between 14 and 2123m (Table 3). To identify habitat suitability and potential limiting factor of *S. minuta*, all ecological variables were analyzed. There were 6/19 ecological variables (Bio 3, Bio 4, Bio 12, Bio 14, Bio 15, Bio 19), which were the most important factors affecting the distribution of *S. minuta* in the study area.

Figure 4 shows a representation of the MaxEnt model for the *S. minuta*. Warmer colors show areas with better predicted conditions. The white dots indicate the presence locations used for training, while the purple dots show the locations of the tests.

Since 1995, leishmaniasis are notifiable diseases in Morocco (Ministerial decree n°683-95). All human cases are listed in leishmaniasis epidemiological information collection system and published yearly. We used data recorded of CL and VL between 2005 and 2016 (28) corresponding to 190 localities.

Statistical analysis of the presence and absence of leishmaniasis cases (cutaneous CL and visceral leishmaniasis VL) and the distribution of *S. minuta* in Morocco showed a positive correlation between VL by *L. infantum* and *S. minuta* abundance ($r= 0.211$) and between CL by *L. tropica* and *S. minuta* abundance ($r= 0.166$).

According to Chi-squared test (χ^2) test, *S. minuta* was more associated to VL distribution

($\chi^2= 7,166$, $ddl= 2$, $P= 0,028$) compared to CL ($\chi^2= 3,256$, $ddl= 2$, $P= 0,196$).

Table 1. Description and sources of environmental variables collected for the model

Environmental variables	Abbreviation	Unit	Source
Annual mean temperature	Bio 1	°C	WorldClim
Mean diurnal range (mean of monthly (max temperature–min temperature))	Bio 2	°C	WorldClim
Isothermality (BIO2/BIO7) (×100)	Bio 3	–	WorldClim
Temperature seasonality (standard deviation ×100)	Bio 4	°C	WorldClim
Max temperature of warmest month	Bio 5	°C	WorldClim
Min temperature of coldest month	Bio 6	°C	WorldClim
Temperature annual range (BIO5–BIO6)	Bio 7	°C	WorldClim
Mean temperature of wettest quarter	Bio 8	°C	WorldClim
Mean temperature of driest quarter	Bio 9	°C	WorldClim
Mean temperature of warmest quarter	Bio 10	°C	WorldClim
Mean temperature of coldest quarter	Bio 11	°C	WorldClim
Annual precipitation	Bio 12	mm	WorldClim
Precipitation of wettest month	Bio 13	mm	WorldClim
Precipitation of driest month	Bio 14	mm	WorldClim
Precipitation seasonality (coefficient of variation)	Bio 15	mm	WorldClim
Precipitation of wettest quarter	Bio 16	mm	WorldClim
Precipitation of driest quarter	Bio 17	mm	WorldClim
Precipitation of warmest quarter	Bio 18	mm	WorldClim
Precipitation of coldest quarter	Bio 19	mm	WorldClim
Elevation	ALT	m	GTOPO30

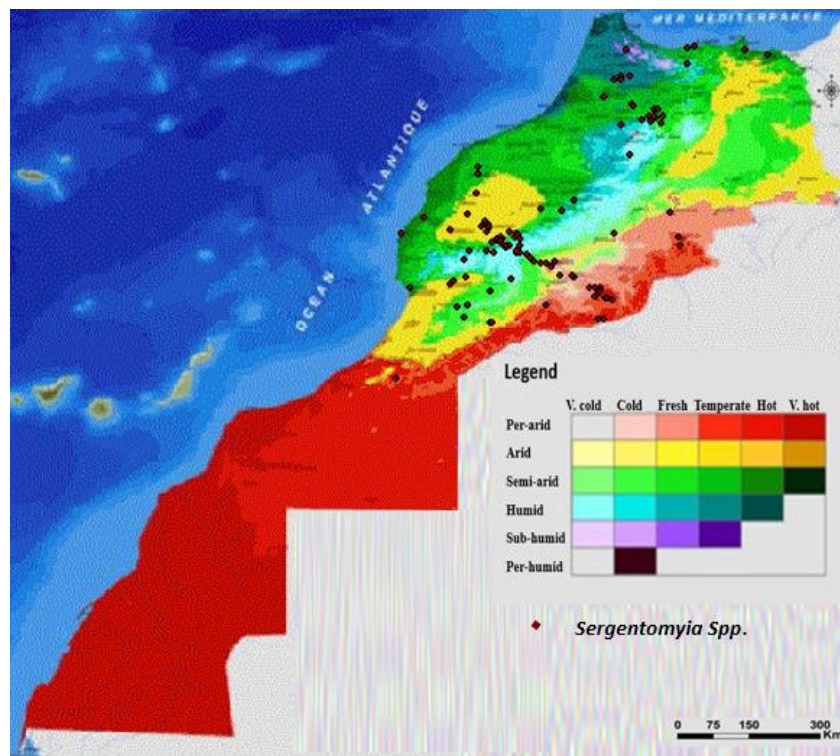


Fig. 1. Distribution of the *Sergentomyia* spp in Morocco according to bioclimate map

Table 2. Analysis of the contribution of the most important ecological variables of the model

Variable	Percent contribution (%)	AUC without the variable	AUC with only the variable
Bio 12	41.2	0.917	0.867
Bio 15	23.9	0.922	0.892
Bio 14	9.4	0.916	0.859
Bio 4	5.9	0.914	0.890
Bio 19	5.5	0.914	0.906
Bio 3	4.8	0.914	0.868

Table 3. Description of *Sergentomyia* species on sampling sites

<i>Sergentomyia</i> spp.	Total of specimens	Number of stations occupied	Altitude range (m)
<i>S. minuta</i>	6746	150	[14–2123]
<i>S. fallax</i>	5582	110	[14–1351]
<i>S. dreyfussi</i>	474	57	[282–1309]
<i>S. africana</i>	43	10	[402–1340]
<i>S. christophersi</i>	99	15	[282–1500]

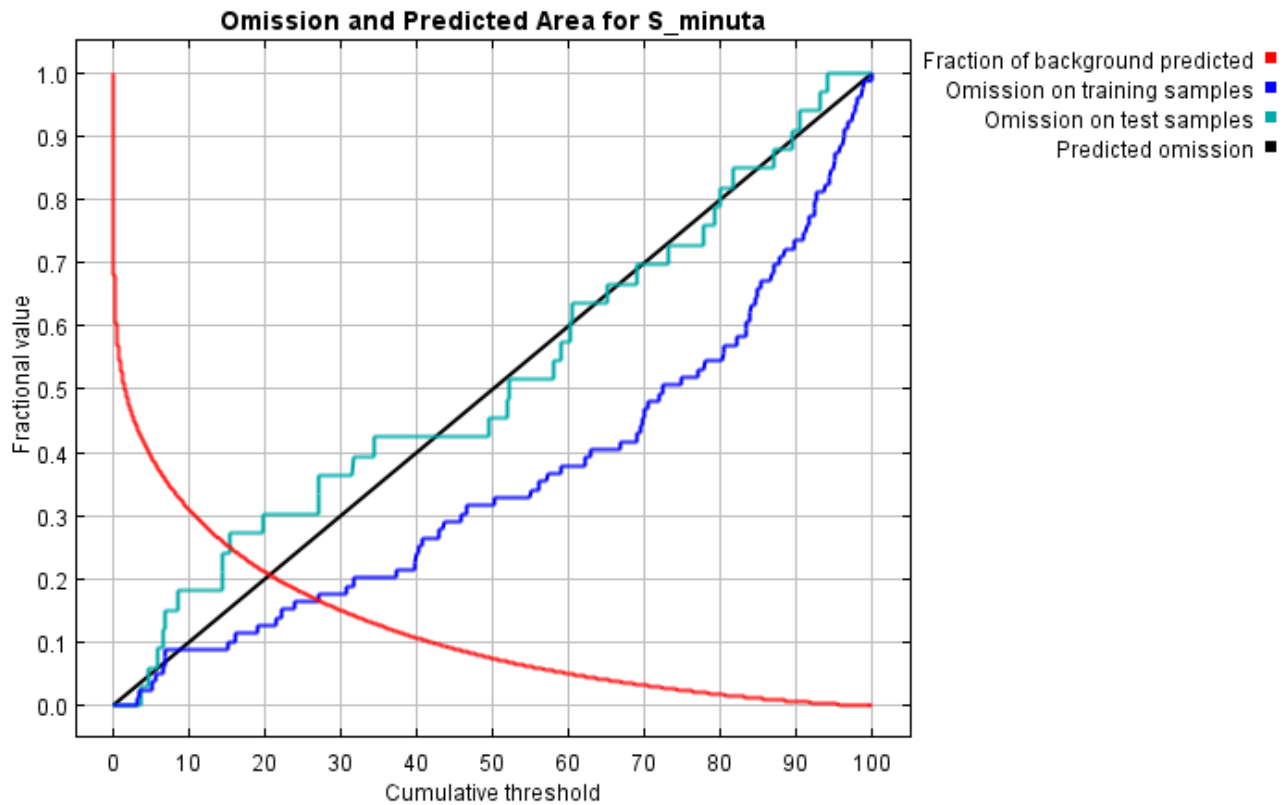


Fig. 2. Analysis of the omission / commission of *Sergentomyia minuta*

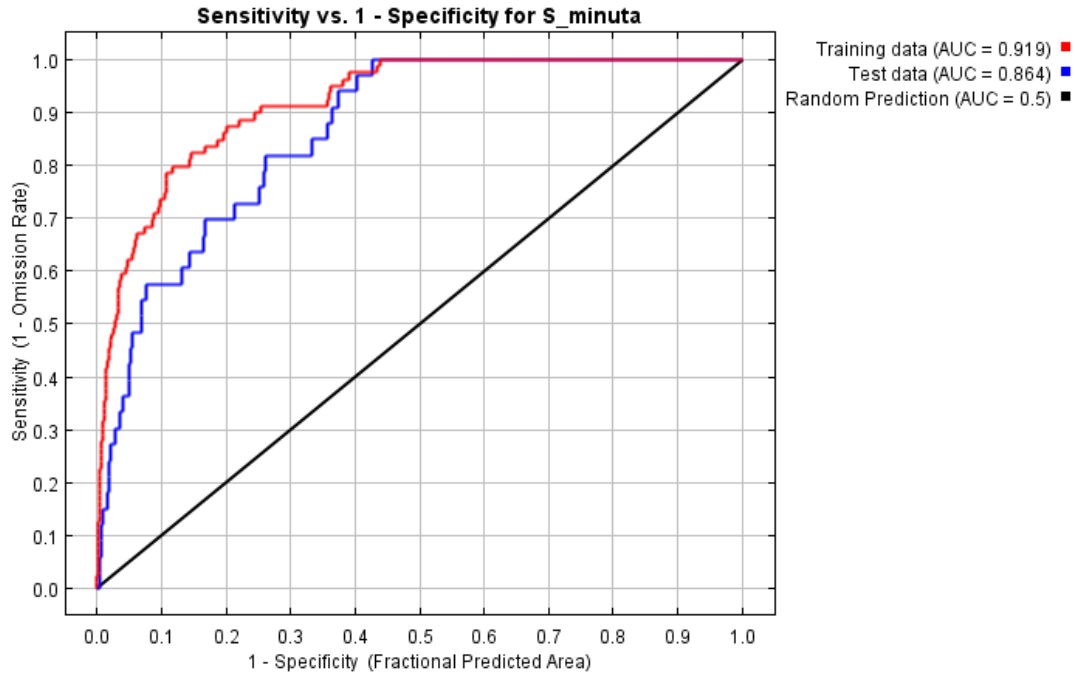


Fig. 3. Sensitivity and performance of the model for *Sergentomyia minuta* species

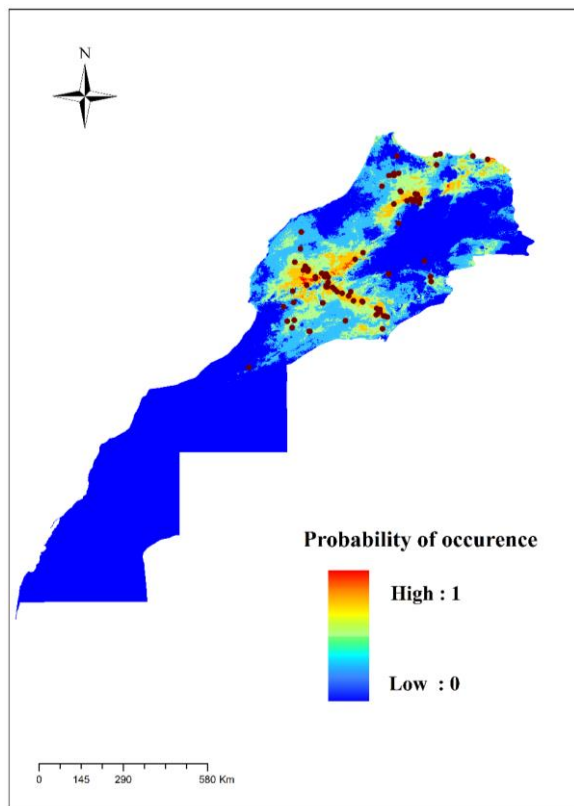


Fig. 4. Estimation of the distribution of *Sergentomyia minuta* species in Morocco by MaxEnt

Discussion

Morocco is located in the subtropical zone. It benefits from a Mediterranean climate, characterized by a dry warm season, nuanced by three essential influences: the progressive remoteness of the Atlantic coast, the altitude and the approach of the southern desert (36). The geographical location of Morocco is a major factor of its bioclimatic diversity which favors the abundance and the diversity of phlebotomine species; including *Sergentomyia* species that can tolerate the aridity (37-39). Thus, a considerable specific richness in Morocco for *Sergentomyia* genus counter to the others Mediterranean's regions such as Tunisia, where only six species of *Sergentomyia* have been reported (40-42).

Sergentomyia species were collected in different environments but are rather subservient to the extradomestic environment (43). This is related to their trophic preferences, and their adaptability to dry and open habitats. Thus, they find suitable conditions in wild environments.

According to WHO (43), sand flies of the genus *Sergentomyia* have long been known to feed on reptiles. They are involved in the transmission of *Sauroleishmania*, of reptiles in the Old World. They do not present an epidemiological risk for humans (44), although some species can feed on humans (45). Recent work on *Sergentomyia* spp shows that these species can be infected by human *Leishmania* spp. and the blood meal analysis shows that it is a mammalian blood, including humans (18). *Leishmania tropica* DNA was detected from *S. ingrami* and *S. hamoni* collected in Ghana (46). All of these results raise questions about the role of *Sergentomyia* spp. as a possible vector of *Leishmania* spp. which has a great impact on human and animal health.

Among all *Sergentomyia* species, *S. minuta* is widely distributed around the Mediterranean area. It is the most abundant *Sergentomyia* species in the present study (Table 3) and early in Morocco (12).

In Greece, the *Sergentomyia* genus represented 84% of the total sand flies fauna with 38% of *S. minuta* (47). In France, Rioux et al. (48) reported a percentage of 73.7% for both genus and species. In Tunisia, it was noted that *Sergentomyia* accounted for 70% of sand flies, of which 41.5% belonged to the species *S. minuta* (49). In Algeria, Belazzoug et al. (50) found this species with a rate of 39.75% of total specimens.

Regarding its potential vector role, Periera et al. (51) have isolated *L. infantum* from *S. minuta*, and blood meal analysis has shown that it is human blood. In Italy, recently, Latrofa et al. (52) detected *L. infantum* DNA in *S. minuta* from endemic area of canine leishmaniasis. *S. minuta* was also naturally infected with *L. major* in Portugal (21) and in Tunisia (53). In addition, Maia et al. (54) detected *Leishmania* spp. in *S. minuta* collected in southern Portugal; while Bravo-Barriga et al. (55) identified *Leishmania* DNA in *S. minuta* of Spain.

In Morocco, though the well-known role of *Phlebotomus papatasi*, *P. sergenti* and *Larrou-*

sus species (*P. perniciosus*, *P. longicuspis* and *P. ariasi*), as competent vector of *L. major*, *L. tropica* and *L. infantum*, respectively (56), that of *S. minuta* needs further investigation.

Ecological niche modelling has been widely used in order to identify the probability of sand fly species occurrence (57). To the best of our knowledge, the present study constitutes a first modeling of *Sergentomyia* species. AUC values of our model were greater than 0.9 (Table 2) indicating that the model performed has a good robustness (31).

In the 190 localities used for the model, we noted the coexistence of *Sergentomyia* species and *Phlebotomus* species including the proven vectors of leishmaniasis forms in Morocco: *P. papatasi*, *P. sergenti*, *P. perniciosus*, *P. ariasi* and *P. longicuspis*. According to the region, *P. sergenti* and *P. papatasi* were more abundant in the central Morocco, while *P. perniciosus* was more recorded in northern Morocco.

The presence and the abundance of *S. minuta* in the endemic area of leishmaniasis in Morocco can be the most important criteria for its possible vectorial incrimination. Our results showed the widespread geographical distribution of *S. minuta* in Morocco, specifically in northern and central Morocco (Fig. 4) where VL and CL foci occur (16, 58).

In addition, a positive correlation between visceral leishmaniasis ($r= 0.211$) and cutaneous leishmaniasis ($r= 0.166$) with *S. minuta* abundance was noted and confirm this *S. minuta*-*Leishmania* spatial overlapping.

Sand fly behaviors can also determine its vector implication. *S. minuta* presented an anthropophilic behavior in Portugal (54). In Morocco, *S. minuta* is a ubiquitous species (13) which shows an adaptation and an important ecological tolerance. It was collected in the different bioclimatic floors with preference to a semiarid climate and altitudes between 800 to 1,000m (12). In addition, the seasonal distribution of *S. minuta* showed abundance from May to September in sub-humid and semi-arid stages in Morocco (15). In arid bioclimate, it

was active during two periods, October–November and April–May–June (59). This long period of *S. minuta* activity favors the *S. minuta-Leishmania* temporal overlapping in these areas.

Sand flies of Morocco, including *Sergentomyia* species, were found highly dependent on environmental conditions; and their abundance and geographical distribution were affected by several ecological variables (59–62). According to our model, annual precipitation (Bio 12) and precipitation seasonality (Bio 15) were significantly more important in the distribution of *S. minuta* in Morocco (Table 2). Precipitation plays an important role in sand flies' life cycle (63). Modeling approach of Chergaf et al. (57), found that annual precipitation has a significant role in distribution of *Leishmania* vector species in Mediterranean basin (57).

S. minuta is recorded in arid region of Tunisia with an annual precipitation between 88 and 157mm (53), also in Portugal with an annual precipitation equal to 730mm (64). In the present study, it prefers regions with an annual precipitation (Bio 12) between: 50–1100mm, and precipitation seasonality (Bio 15) between: 30–100mm (Supporting Information S1).

Conclusion

Effective vector control requires accurate vector identification. The spatial and temporal overlapping of *S. minuta* with *Leishmania* species seemed to present the important criteria necessary to be incriminated as a potential vector of the *Leishmania* species in Morocco. Hence, the present study opened a debate on the potential role of *Sergentomyia* species, especially *S. minuta* in the transmission of leishmaniasis in Morocco. Hypothesis deserves further researches including, but not limited to, protozoan isolation from engorged specimens as well as experimental transmission.

Acknowledgements

The authors would like to thank Elouise

Gaylard for linguistic consultation. The authors declare that there is no conflict of interest.

References

1. Hazratian T, Vatandoost H, Oshaghi MA, Yaghoobi-Ershadi MR, Fallah E, Rafizadeh S, Shirzadi MR, Shayeghi M, Akbarzadeh K, Rassi Y (2016) Diversity of sand flies (Diptera: Psychodidae) in endemic focus of visceral leishmaniasis in Azar Shahr District, East Azarbaijan Province, north west of Iran. *J Arthropod Borne Dis.* 10(3): 328–334.
2. Belkaid Y, Valenzuela JG, Kamhawi S, Rowton E, Sacks DL, Ribeiro JM (2000) Delayed-type hypersensitivity to *Phlebotomus papatasi* sand fly bite: An adaptive response induced by the fly?. *Proc Natl Acad Sci USA.* 97(12): 6704–6709.
3. Killick-Kendrick R, Killick-Kendrick M (1999) Biology of sand fly vectors of Mediterranean canine leishmaniasis. In: *Canine Leishmaniasis: an update. The First International Canine Leishmaniasis Forum, 1999 February, Barcelona, Spain*, pp. 28–31.
4. Mukherjee S, Hassan MQ, Ghosh A, Ghosh KN, Bhattacharya A, Adhya S (1997) *Leishmania* DNA in *Phlebotomus* and *Sergentomyia* species during a kala-azar epidemic. *Am J Trop Med Hyg.* 57: 423–425.
5. Berdjane-Brouk Z, Koné AK, Djimde AA, Charrel RN, Ravel C, Delaunay P, del Giudice P, Diarra AZ, Doumbo S, Goita S, Thera MA, Depaquit J, Marty P, Doumbo OK, Izri A (2012) First detection of *Leishmania major* DNA in *Sergentomyia* (Spelaemyia) *darlingi* from cutaneous leishmaniasis foci in Mali. *PLoS One.* 7: e28266.
6. Kahime K, Boussaa S, Bounoua L, Ouanaimi F, Messouli M, Boumezzough A (2014) Leishmaniasis in Morocco: diseases and vectors. *Asian Pac J Trop Dis.* 4: 530–534.

7. Rioux JA, Guilvard E, Dereure J, Lanotte G, Denial M, Pratlong F, Serres E, Belmonte A (1986) Infestation naturelle de *Phlebotomus papatasi* (Scopoli, 1786) par *Leishmania major* MON-25. A propos de 28 souches isolées dans un foyer du Sud Marocain. In Rioux J.A. (ed.) *Leishmania*. Taxinomie et phylogénèse. Applications écoépidémiologiques. International Colloquium CNRS/INSERM, 1984 July 2–6. Institut Méditerranéen d'Etudes Épidémiologiques et Ecologiques, Montpellier, France, pp. 471–480.
8. Ajaoud M, Es-sette N, Charrel RN, laamrani-Idrissi A, Nhammi H, Riyad M, Lemrani M (2015) *Phlebotomus sergenti* in a cutaneous leishmaniasis focus in Azilal Province (High Atlas, Morocco): Molecular detection and genotyping of *Leishmania tropica* and feeding behavior. PLoS Negl Trop Dis. 9: e0003687.
9. Zarrouk A, Kahime K, Boussaa S, Belqat B (2015) Ecological and epidemiological status of species of the *Phlebotomus perniciosus* complex (Diptera: Psychodidae, Phlebotominae) in Morocco. Parasitol Res. 115(3): 1045–1051.
10. Moroccan Ministry of Health (2010) Fight against Leishmaniasis. Activity Guide. Directorate of Epidemiology and Disease Control, Parasitic Diseases Service, Ministry of Health. Morocco. Available at: <http://www.sante.gov.ma>
11. Ouanaimi F, Boussaa S, Boumezzough A (2015) Phlebotomine sand flies (Diptera: Psychodidae) of Morocco: results of an entomological survey along three transects from northern to southern country. Asian Pac J Trop Dis. 5(4): 299–306.
12. Kahime K, Boussaa S, El Mazabi A, Boumezzough A (2015) Spatial relations among environmental factors and phlebotomine sand fly populations (Diptera: Psychodidae) in central and southern Morocco. J Vector Ecol. 40(2): 342–354.
13. Boussaa S, Boumezzough A, Sibold B, Alves-Pires C, Morillas Marquez F, Glasser N, Pesson B (2009) Phlebotomine sandflies (Diptera: Psychodidae) of the genus *Sergentomyia* in Marrakech region, Morocco. Parasitol Res. 104: 1027–1033.
14. Guernaoui S, Boumezzough A, Laamrani A (2006) Altitudinal structuring of sandflies (Diptera: Psychodidae) in the High-Atlas mountains (Morocco) and its relation to the risk of leishmaniasis transmission. Acta Trop. 97: 346–351.
15. Bailly-Choumara H, Abonnenc E, Pastre J (1971) Contribution à l'étude des phlébotomes du Maroc. (Diptera: Psychodidae): données faunistiques et écologiques. Cah ORSTOM. Ser Entomol Med Parasitol. 9: 431–460.
16. Boussaa S, Guernaoui S, Pesson B, Boumezzough A (2005) Seasonal fluctuations of phlebotomine sand fly populations (Diptera: Psychodidae) in the urban area of Marrakech, Morocco. Acta Trop. 95: 86–91.
17. El Miri H, Rhajaoui M, Himmi O, Ouahabi S, Benhoussa A, Faraj C (2013) Entomological study of five cutaneous leishmaniasis foci in the Sidi Kacem Province, north Morocco. Ann Soc Entomol Fr. 49 (2): 154–159.
18. Maia C, Depaquit J (2016) Can *Sergentomyia* (Diptera: Psychodidae) play a role in the transmission of mammal-infecting *Leishmania*? Parasite. 23: 55.
19. Parvizi P, Amirkhani A (2008) Mitochondrial DNA characterization of *Sergentomyia sintoni* populations and finding mammalian *Leishmania* infections in this sand fly by using ITSrDNA. Iran J Vet Res. 22: 9–18.
20. Berdjane-Brouk Z, Koné AK, Djimde AA, Charrel RN, Ravel C, Delaunay P, del Giudice, P, Diarra AZ, Doumbo S, Goita S, Thera MA, Depaquit J, Marty P, Doumbo OK, Izri A (2012) First detection of *Leishmania major* DNA in *Ser-*

- gentomyia* (Spelaeomyia) *darlingi* from cutaneous leishmaniasis foci in Mali. PLoS One. 7: e28266.
21. Campino L, Cortes S, Dionísio L, Neto L, Afonso MO, Maia C (2013) The first detection of *Leishmania major* in naturally infected *Sergentomyia minuta* in Portugal. Mem Inst Oswaldo Cruz. 108: 516–518.
 22. Mutinga MJ, Massamba NN, Basimike M, Kamau CC, Amimo FA, Onyido AE, Omogo DM, Kyai FM, Wachira DW (1994) Cutaneous leishmaniasis in Kenya: *Sergentomyia garnhami* (Diptera: Psychodidae), a possible vector of *Leishmania major* in Kitui District: a new focus of the disease. East Afr Med J. 71: 424–428.
 23. Mukherjee S, Hassan MQ, Ghosh A, Ghosh KN, Bhattacharya A, Adhya S (1997) *Leishmania* DNA in *Phlebotomus* and *Sergentomyia* species during a kala-azar epidemic. Am J Trop Med Hyg. 57: 423–425.
 24. Kanjanopas K, Siripattanapipong S, Ninsaeng U, Hitakarun A, Jitkaew S, Kaewtaphaya P, Tanariya P, Mungthin M, Charoenwong C, Leelayoova S (2013) *Sergentomyia* (*Neophlebotomus*) *gemmea*, a potential vector of *Leishmania siamensis* in southern Thailand. BMC Infect Dis. 13: 333.
 25. Ba Y, Trouillet J, Thonnon J, Fontenille D (1999) *Phlebotomus* of Senegal: survey of the fauna in the region of Kedougou. Isolation of arbovirus. Bull Soc Pathol Exot. 92(2): 131–135.
 26. Charrel RN, Izri A, Temmam S, de Lamballerie X, Parola P (2006) Toscana Virus RNA in *Sergentomyia minuta* Flies. Emerg Infect Dis. 12(8): 1299–1300.
 27. Geevarghese G, Arankalle VA, Jadi R, Kanojia PC, Joshi MV, Mishra AC (2005) Detection of Chandipura virus from sand flies in the genus *Sergentomyia* (Diptera: Phlebotomidae) at Karimnagar District, Andhra Pradesh, India. J Med Entomol. 42: 495–496.
 28. Kholoud K, Denis S, Lahouari B, Abdelmonaim M, Hidan EJ, Soua B (2018) Management of Leishmaniasis in the Era of Climate Change in Morocco. Int J Environ Res Public Health. 15(7): 1542.
 29. High Planning Commission of Morocco (2014) Report of General Census of Population and Housing. Morocco. Available at: <http://www.hcp.ma>
 30. Hijmans R, Cameron S, Parra J, Jones P, Jarvis A, Richardson K (2005) WorldClim, Version 1.3. University of California, Berkeley.
 31. Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecol Modell. 190: 231–259.
 32. Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, McC J, Overton M, Townsend Peterson A, Phillips SJ, Richardson K, Scachetti-Pereira R, Schapire RE, Soberón J, Williams S, Wisz MS, Zimmermann NE (2006) Novel methods improve prediction of species distributions from occurrence data. Ecography. 29: 129–151.
 33. Pearson TRH, Brown SL, Birdsey RA (2007) Measurement guidelines for the sequestration of forest carbon Gen. Tech. Rep. NRS-18. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. p. 42.
 34. Nogués-Bravo D (2009) Predicting the past distribution of species climatic niches. Glob Ecol Biogeogr. pp. 521–531.
 35. Mokhtari N, Mrabet R, Lebailly P, Bock L (2014) Spatialisation des bioclimats, de l'aridité et des étages de végétation du Maroc. Rev Mar Sci Agron Vét. 2(1): 50–66.
 36. Sebbar A (2013) Etude de la variabilité et de l'évolution de la pluviométrie au Ma

- roc (1935–2005): Réactualisation de la carte des précipitations. [PhD dissertation]. Faculté des Sciences Ben M'Sik Casablanca, Morocco.
37. Adler S, Theodor O (1929) The distribution of sandflies and leishmaniasis in Palestine, Syria and Mesopotamia. *Ann Trop Med Parasitol.* 23: 269–306.
 38. Lupasco G, Duport M, Dancesco P, Cristesco A (1965) Recherches sur les espèces de Phlébotomes sauvages de Roumanie. *Arch Roum Pathol Exp Microbiol.* 24: 195–202.
 39. Newstead R, Sinton JA (1921) On a collection of Pappataci flies (*Phlebotomus*) from India. *Ann Trop Med Parasitol.* 15: 103–106.
 40. Depaquit J, Léger N, Killick-Kendrick R (1998) Description de *Phlebotomus (Paraphlebotomus) riouxi* n. sp (Diptera: Psychodidae) d'Afrique du Nord. *Parasite.* 151–158.
 41. Ghrab J, Rhim A, Bach-Hamba D, Chahed MK, Aoun K, Noura S, Bouratbine A (2006) Phlebotominae (Diptera: Psychodidae) of human leishmaniasis sites in Tunisia. *Parasite.* 13 : 23–33.
 42. Chamkhi J, Guerbouj S, Ben Ismail R, Guizani I (2006) Description of the hitherto unknown female of *Phlebotomus (Larrousius) Chadlii*, Rioux, Juminer and Gibily, 1966 (Diptera: Psychodidae). *Parasite.* 13 (4): 299–303.
 43. World Health Organization (2002) Urbanization: an increasing risk factor for leishmaniasis. *Wkly Epidemiol Rec.* 77: 365–372.
 44. Lane RP (1993) Sandflies (Phlebotominae). In: Lane, RP and Crosskey, RW (Eds.), *Medical insects and arachids.* (1st Edn.), London, Chapman and Hall. pp. 78–119.
 45. Hoogstraal H, Dietlein DR, Heyneman D (1962) Leishmaniasis in the Sudan Republic. Preliminary observations on man-biting sandflies (Psychodidae: *Phlebotomus*) in certain Upper Nile endemic Nile endemic areas. *Trans R Soc Trop Med Hyg.* 411–422.
 46. Nzelu CO, Kato H, Puplampu N, Desewu K, Odoom S, Wilson MD, Sakurai T, Katakura K, Boakye DA (2014) First detection of *Leishmania tropica* DNA and *Trypanosoma* species in *Sergentomyia* sand flies (Diptera: Psychodidae) from an outbreak area of cutaneous leishmaniasis in Ghana. *PLoS Negl Trop Dis.* 8 (2): e2630.
 47. Madulo-leblond G (1983) Les phlébotomes (Diptera: Phlebotomidae) des îles Ioniennes. [PhD dissertation]. Université de Reims, France.
 48. Rioux JA, Lanotte G, Petter F, Dereure J, Akalay O, Pralong F, Velez ID, Fikri NB, Maazoum R, Denial M, Jarry DM, Zahaf A, Ashford RW, Cadi-Soussi M, Killick-Kendrick R, Benmansour N, Moreno G, Perieres J, Guilvard E, Zribi M, Kennou MF, Rispail P, Knechtli E, Serres E (1986) Les leishmanioses cutanées du bassin méditerranéen occidental: de l'identification enzymatique à l'analyse éco-épidémiologique, l'exemple de trois 'foyers', tunisien, marocain et français. In: *Leishmania.* Taxonomie et Phylogénèse. Applications Écoépidémiologiques, ed. Rioux, J.A. Montpellier, France: Institut Méditerranéen d'Études Épidémiologiques et Ecologiques. pp. 365–395.
 49. Haddad N (1998) Les phlébotomes du Liban-Inventaire et corollaire éco-épidémiologique. Université de Pharmacie Reims, France.
 50. Belazzoug S, Mahzoul D, Rioux JA (1986) Les phlébotomes (Diptera: Psychodidae) de M'sila et Bousaada. *Arch Inst Pasteur Alger.* 55: 117–124.
 51. Peira S, Pita-Pereira D, Araujo-Pereira T, Britto C, Costa-Rego T, Ferrolho J, Vilhena M, Rangel EF, Vilela ML, Afonso MO (2017) First molecular detection of *Leishmania infantum* in *Sergentomyia minuta*

- (Diptera: Psychodidae) in Alentejo, southern Portugal. *Acta Trop.* 174 : 45–48.
52. Latrofa MS, Iatta R, Dantas-Torres F, Annoscia G, Gabrielli S, Pombi M, Grandoni L, Otranto D (2018) Detection of *Leishmania infantum* DNA in phlebotomine sand flies from an area where canine leishmaniosis is endemic in southern Italy. *Vet Parasitol.* 253: 39–42.
 53. Jaouadi K, Ghawar W, Salem S, Gharbi M, Bettaieb J, Yazidi R, Harrabi M, Hamarsheh O, Ben Salah A (2015) First report of naturally infected *Sergentomyia minuta* with *Leishmania major* in Tunisia. *Parasit Vectors.* 8: 649.
 54. Maia C, Parreira R, Cristóvão JM, Freitas FB, Afonso MO, Campino L (2015) Molecular detection of *Leishmania* DNA and identification of blood meals in wild caught phlebotomine sand flies (Diptera: Psychodidae) from southern Portugal. *Parasit Vectors.* 8: 173.
 55. Bravo-Barriga D, Parreira R, Maia C, Blanco-Ciudad J, Odete AM, Frontera E, Campino L, Pérez-Martín JE, Serrano Aguilera FJ, Reina D (2016) First molecular detection of *Leishmania tarentolae*-like DNA in *Sergentomyia minuta* in Spain. *Parasitol Res.* 115: 1339.
 56. Mhaidi I, El Kacem S, Ait Kbaich M, El Hamouchi A, Sarih M, Akarid K, Lemrani M (2018) Molecular identification of *Leishmania* infection in the most relevant sand fly species and in patient skin samples from a cutaneous leishmaniasis focus, in Morocco. *PLoS Negl Trop Dis.* 12 (3): e0006315.
 57. Chalhaf B, Chemkhi J, Mayala B, Harrabi M, Benie GB, Michael E, Ben Salah A (2018) Ecological niche modeling predicting the potential distribution of *Leishmania* vectors in the Mediterranean basin: impact of climate change. *Parasit Vectors.* 11(1): 461.
 58. Rhajaoui M (2011) Human leishmaniasis in Morocco. A nosogeographical diversity. *Pathol Bio.* 59(4): 226–229.
 59. Rioux JA, Rispail P, Lanotte G, Lepart J (1984) Relations Phlébotomes-bioclimats en écologie des leishmanioses Corollaires épidémiologiques. L'exemple du Maroc. *Bull Soc Bot. France.* 131(2–4): 549–557.
 60. Rispail P, Dereure J, Jarry D (2002) Risk zones of human Leishmaniasis in the Western Mediterranean basin: correlations between vector sand flies, bioclimatology and phytosociology. *Mem Inst Oswaldo Cruz.* 97(4): 477–483.
 61. Bounoua L, Kahime K, Houti L, Blakey T, Ebi KL, Zhang P, Imhoff ML, Thome KJ, Dudek C, Sahabi SA, Messouli M, Makhoul B, El Laamrani A, Boumezzough A (2013) Linking climate to incidence of zoonotic cutaneous leishmaniasis (*L. major*) in pre-Saharan North Africa. *Int J Environ Res Public Health.* 10(8): 3172–3191.
 62. Boussaa S, Kahime K, Samy AM, Salem AB, Boumezzough A (2016) Species composition of sand flies and bionomics of *Phlebotomus papatasi* and *P. sergenti* (Diptera: Psychodidae) in cutaneous leishmaniasis endemic foci, Morocco. *Parasit Vectors.* 9: 60.
 63. Kasap OE, Alten B (2006) Comparative demography of the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae) at constant temperatures. *J Vector Ecol.* 31: 378–385.
 64. Branco S, Alves-Pires C, Maia C, Cortes S, Cristóvão JMS, Gonçalves L, Campino L, Afonso MO (2013) Entomological and ecological studies in a new potential zoonotic leishmaniasis focus in Torres Novas municipality, Central Region, Portugal. *Acta Trop.* 125(3): 339–348.