

**Taxonomic and Biodiversity Study of Sandfly Species in the Fez Region, Central Morocco**Najoua Darkaoui¹, Abdellatif J. Idrissi¹, Fatima Z. Talbi^{1,2*}, Youness El Fattouhi¹, Abdelkarim Taam³, Nordine Nouayti⁴, Moussa Benboubker⁵, Abdelhakim E.O. Lalami^{1,6}¹ Sidi Mohamed Ben Abdellah University, Faculty of Sciences Dhar El Mahraz, Laboratory of Biotechnology, Conservation and Valorization of Natural Resources (LBCVNR), 30000 Fez, Morocco² Hassan First University of Settat, Faculty of Sciences and Technologies, Laboratory of Biochemistry, Neurosciences, Natural Resources and Environment, P.O. Box 577, 26000 Settat, Morocco³ Laboratory of Engineering Sciences, National School of Applied Sciences (ENSA), Ibn Tofail University, Morocco⁴ Applied Sciences Laboratory, Water and Environmental Engineering Team. National School of Applied Sciences, Al Hoceima, Abdelmalek Essaadi University, Tanger-Tétouan-AL Hoceima, Larache, Morocco⁵ Human Pathology Bio-Health and Environment Laboratory, Faculty of Medicine and Pharmacy, Sidi Mohammed Ben Abdellah University, Fez, Morocco⁶ Higher Institute of Nursing Professions and Health Techniques of Fez, Regional Health Directorate Fez-Meknes, EL Ghassani Hospital, 30000 Fez, Morocco

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ABSTRACT

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Leishmaniasis is a parasitic disease caused by infectious protozoa of the genus *Leishmania* that are transmitted by the bite of phlebotomine sandflies. However, there has not been any taxonomic study on sandfly biodiversity in the Fez Province. This study was aimed at identifying and making an inventory of the most common sandflies in the different regions of the Fez Province. Sandfly collection was done twice a month with sticky traps from May 2017 to April 2018. Spatial distribution analysis was performed using Qgis 2.18 software. A total of 816 sandflies were collected, with a male predominance (sex ratio of 20.47) and divided into two genera: *Phlebotomus* (46.82%) and *Sergentomyia* (53.18%). The predominant species of the *Phlebotomus* genus were *P. sergenti* (26.96%), followed by *P. perniciosus* (10.78%), and *P. papatasi* (9.07%). *Sergentomyia minuta* was the most dominant species compared to the other specimens with a proportion of (44.24%) followed by *S. fallax* (7.97%), and *S. antennata* (0.98%). GIS analysis revealed that Ain Nokbi, Boujloud, Dhar Richa, and Zlilig are likely to be sandfly biotopes. The prevalence of *Phlebotomus* species with a large distribution area in the prefecture of Fez and high population numbers could explain the reasons for the cutaneous leishmaniasis observed in the study period. These observations implicate Fez Province as having a significant risk of leishmaniasis transmission. The findings of this study can assist in implementing appropriate control measures for Fez regions by developing a comprehensive leishmaniasis risk map.

Keywords: Biodiversity, Central Morocco, Cutaneous leishmaniasis, Sandfly, Fez, Taxonomic.

Introduction

Leishmaniasis is a parasitic disease caused by infectious protozoa of the genus *Leishmania*, that affects a wide range of mammals, including humans and dogs.¹ It is transmitted between vertebrates by a blood-sucking phlebotomine sandfly.¹ Visceral leishmaniasis (VL) caused by *L. infantum*, is transmitted by *Phlebotomus longicuspis*, *P. perniciosus*, and *P. ariasi*, while the skin forms are caused by *L. tropica* and are transmitted by *P. sergenti*. The diffused skin pathogen, *L. major* is transmitted by *P. papatasi*.¹ The disease is endemic to 88 countries and almost all the continents, namely Africa, Central and South America, Asia, and Europe, except North America and Australia.^{2,3} Leishmaniasis is one of the most rapidly spreading infections, posing a serious threat to public health. It is the second most common cause of parasitic morbidity after malaria.⁴

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Visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL) are the three clinical entities of leishmaniasis.⁵ Each year, 500,000 new cases of VL are reported. There are an estimated 12 million cases of various forms of leishmaniasis worldwide, with a yearly incidence of 1.5 to 2 million new diagnoses. Only one-third of new cases are reported to the authorities.^{4,6} Cutaneous leishmaniasis is the most common form, which produces ulcers on exposed body parts and leaves permanent scars. The distribution and number of sandfly species in Morocco are influenced by climatic and geographical diversity.^{7,8,9} Therefore, leishmaniasis continues to pose a serious public health problem.¹⁰⁻¹² There is an annual emergence of new foci of leishmaniasis. The Ministry of Health documented 4,426 and 2,990 cases in 2011 and 2012, with 4.92% and 7.19%, respectively, in the region of Fes-Meknes. The first outbreak of CL registered in the Fez-Meknes region was in 2001, with 1,600 cases only in the province of Moulay Yaâcoub.^{4,6,11,13,14} Twenty species of *Leishmania* are human pathogens,¹⁵ and more than 800 species of sandflies are currently recognized worldwide. Around 10% of them have been identified as leishmaniasis vectors. However, the vectorial capacity of only 30 species has been confirmed.^{16,17} Three were discovered in the city of Fez, accounting for 10% of the pathogenic species recorded. In Morocco, there are 23 species of sandflies, although only five of the *Phlebotomus* genus are verified leishmaniasis carriers.¹⁸ In the Fez-Meknes region of central Morocco, some studies were conducted by

Talbi *et al.* between 2015 and 2016 in the locality of Aichoun (Sefrou Province),¹⁹ and Lahouiti *et al.* in Moulay Yacoub Province between 2014 and 2016, successively.¹³ Also, research carried out in the province of Meknes by El Omari *et al.* from March 2016 until April 2017 contributed to the bulk of the information available on leishmaniasis.²⁰ However, it should be noted that no work on the taxonomic study of the phlebotomine biodiversity has been carried out in Fez province. The present study was conducted to identify and make an inventory of the main sandflies in the various stations of Fez Province, thereby contributing to the future design of the Fez-Meknes region's leishmaniasis risk map and reinforcing surveillance strategies.

Materials and Methods

Study area

Fez is located in northern Morocco (34° 03' 00" N, 4° 58' 59" W) at an elevation of 406 m (Figure 1). It covers an area of 332 km² and has a population of 1,150,131 inhabitants, with an urban population of 1,129,768 inhabitants, and an estimated rural population of 20,363 inhabitants. The population density in Fez is 3,464 inhabitants/km² with an average annual growth rate of 1.84%, according to the data from the General Census of Population and Housing (GPHC) conducted in September 2014 (High Commission for Planning, 2014). The region is divided into nine cities (provinces), which include Fez-Meknes, Moulay Yacoub, Sefrou, El hajeb, Boulemane, Ifrane, Taza, and Taounate. According to Emberger,⁷ the City of Fez belongs to the semi-arid Mediterranean bioclimatic stage characterized by a hot climate in summer and a cold climate in winter. The annual rainfall pattern has two distinct periods, one rainy from October to April and another dry from June to September. In general, the relief of Fez is composed of the high plain of Saïs and the low valley of Sebou and between the two, a narrow valley ensures the passage with more than 200 m of gradient.⁹

Collection of sandflies

Several factors were taken into account when choosing the sandfly proliferation biotopes (laying and resting media). The geographical features, altitudes, geology of the region, environmental and sanitary conditions (proximity to uncontrolled public dumps, manure, etc.), dilapidated houses, caves, and wall cracks are the main factors for phlebotomy proliferation, according to the results of a sociological survey conducted by the health services related to the leishmaniasis case inventory in the region (period 2016-2017). Using these criteria, the study stations of choice included Ain Nokbi (34°06'44"N,4°95'75"W), Dhar Richa (34°07'13"N,4°98'28"W), Boujloud (34°06'37"N,4°98'53"W), Cotef (34°06'65"N,4°98'23"W), and Zillig (33°96'16"N,5°08'80"W). The study sites and their locations are presented in Table 1. From May 2017 to April 2018, the above stations were surveyed regularly. Sandflies were captured using sticky traps put out in the late afternoon at several urban and peri-urban settings during each prospecting excursion. The following morning, a quick examination was conducted to better understand the region's specific characteristics.

Sandfly sampling

The traps were designed using square sheets of white paper (21 x 29.7 cm) saturated with castor oil and arranged in a cone-shaped form (in cylindrical cavities) with a thread in an upright position to keep them in place. Their transparency implies that they have been impregnated with oil. During the trapping period, each site had eight sticky traps deployed (each station includes at least 3 sites). The impregnated sheets were collected at about 8 a.m. and placed in an envelope with the station number, the biotope, the number of sticky traps put and retrieved, and the date of the periodic visits (Figure 2).

Dissection and identification of sandflies

The collected sandflies were washed in sterile distilled water before being stored in 70% alcohol until they were assembled. They were then cleaned with 10% potash for 4 hours and Marc-André liquid for 2 hours.³ The cibarium armature, penile valve shape, number and position of spines on genitalia, number of apical bristles on coxite in males, and the pharyngeal armature, spermatheca, and shape as well as the number of cibarium teeth in females were used to identify the species.²¹⁻²³

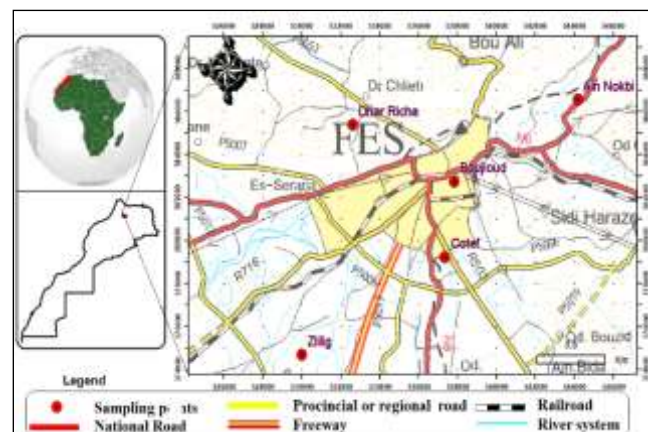


Figure 1: Map of the geographical location of the prefecture of Fez.

Table 1: Description and characteristics of phlebotomy trapping sites.

Site	Nature of biotope	Hygiene state
Dhar Richa	Caves and stables	Manure, waste of all kinds
Boujloud	Wall and caves	Waste
Ain Nokbi	Ruined house	Waste of all kinds
Cotef	Ruined house (old textile complex)	Industrial waste, waste of all kinds
Zillig	Caves	Manure, waste of all kinds



Figure 2: Attachment of the adhesive traps. A: Trap using a string; B: Trap installed in a cone

Females were dissected in distilled water with a magnifying glass, with the head and genitalia on lamella in a drop of Marc-André's solution. The males were mounted directly on lamella, and the assemblages were inspected for species identification under a light microscope (objectives x10 and x40). According to the Leishmaniasis Control Activities guide, the sandfly identify was compared with the sandfly determination key used in Morocco.¹¹

Processing and analysis of data

Statgraphics version 18 and GIS were used to process the topographic data. Five characteristics of the phlebotomy fauna were identified. The relative abundance for a given species was calculated as the ratio of the number of individuals trapped to the total number of individuals in the stand.⁸

$$AR = (N_i / N) \times 100$$

Where N_i is the number of species i , and N is the total number of sandflies collected.

The density is expressed by the number of traps captured per area (m²) and per night of capture.

$$\text{Trapped area} = \text{Trap area} \times \text{Number of traps used} \times 2$$

The sex ratio represents the number of males/females. The specific richness corresponds to the total number of species recorded at the various stations surveyed during the study period. The frequency of occurrence was calculated according to the following formula:

Common species are present in 50% or more of the surveys carried out; accessory species are present in 25 to 49% of the samples; rare species whose frequency is less than 25% and more than 10%; Very rare species qualified as sporadic have a frequency of less than 10%.⁸
 $C = r / R \times 100$

Where C is the frequency of occurrence of the species, r is the total number of samples containing the species, and R is the total number of samples collected.

Qgis 2.18 software was used to investigate the distribution of sandfly species by integrating data on leishmaniasis into a geographic information system.

Results and Discussion

Taxonomic study of sandflies

During the trapping period, 816 sandflies were captured, representing two genera, *Phlebotomus* and *Sergentomyia*. Only 382 individuals (46.82%) of the genus *Phlebotomus* sandflies were found, whereas 434 individuals of the genus *Sergentomyia* were obtained (53.18%). In the first investigation in Morocco, Boussaa *et al.* discovered a sandfly population dominated by members of the genus *Sergentomyia*.²⁴ In France, the studies of Rioux *et al.* revealed that the genus *Sergentomyia* exists in 73% of cases, while Haddad,²⁵ discovered it in 97.62% of cases in Lebanon. The results of this study are in agreement with other findings such as those carried out in Algeria by Bounamous,²⁶ which also revealed the occurrence of this genus with the three species that were found in this study. The high percentage of the genus *Sergentomyia* was then consistent with bibliographical data from faunistic inventories conducted in the Mediterranean region. These high percentages are explained by the expansion of the distribution area of the genus *Sergentomyia* and the herpetophilic behaviour of the species of this genus, which seek their prey in the crevices where the oiled papers were placed.

Concerning *Phlebotomus* of medical importance, with 220 specimens detected at a 14.69 ph/m² density, *P. sergenti* was the most prominent genus, followed by 88 specimens of *P. perniciosus* at a 5.88 ph/m² and 74 specimens of *P. papatasi* at a 4.94 ph/m² density. With 361 individuals and a 24.11 ph/m², *S. minuta* was the most frequently collected sandfly during the study period, followed by *S. fallax* with 65 individuals and a 4.34 ph/m², and finally, *S. antennata* with only 8 specimens and a 0.53 ph/m². In terms of sex ratio, the results show that there was a predominance of males over females (778 males and 38 females). In general, comparing sex ratios by species and adhesive traps reveals that there were more males than females in all species (Table 2). *Phlebotomus* was represented by three species: *P. sergenti* (26.96%), followed by *P. perniciosus* (10.79%), and *P. papatasi* (9.07%). Similarly, the second genus, *Sergentomyia*, was represented by three species: *S. minuta* which was the dominant species (44.24%), *S. fallax* (7.96%), and *S. antennata* (0.98%). Only 382 individuals (46.82%) of *Phlebotomus* sandflies were present, while 434 individuals of *Sergentomyia* sandflies were present (53.18%). The

findings of research conducted in the Sefrou Province⁶ and the Moulay Yaacoub Province,¹⁴ on the other hand, revealed the presence of both genera, with *Phlebotomus* predominating over *Sergentomyia*. *Phlebotomus* sandflies were represented by only 382 individuals (46.82%), while 434 individuals (53.18%) of *Sergentomyia* sandflies were present. On the contrary, the findings of research conducted in the Sefrou,⁶ and Moulay Yaacoub,¹⁴ provinces revealed the presence of the two genera, with the genus *Phlebotomus* predominant over *Sergentomyia*.

The findings of a survey conducted from May 2017 to April 2018 allowed for the classification of six species at various locations in Fez (Figures 3, 4, 5, 6, 7). When male sandflies (95.34%) of each species were compared to female sandflies (4.66%), the males were dominating. The latter were classified according to their physiological states, such as whether they were not engorged, engorged, or gravid. Additionally, the sex ratio of 20.47 shows that there is a dominance of males over females (778 of 816 are males). This observation can be explained by the fact that males prefer to rest near roosts, while females travel in search of a blood meal or an egg-laying bed.²⁷ *Phlebotomus sergenti* was the most frequently collected species in the genus *Phlebotomus*, with a frequency of 57.6% at the city level of Fez. *Phlebotomus sergenti* requires very special medical attention.¹⁵ It is the only proven vector of *L. tropica* in Morocco and the main vector in Mediterranean countries.^{28,29} *Phlebotomus sergenti* can spread the disease through bites and feeds on people in the wild.³⁰ Its significance as a vector has been demonstrated in several countries, including Saudi Arabia,²⁸ Morocco, the Middle East,³¹ and Ethiopia.³² *Phlebotomus sergenti* was described for the first time in Morocco by Delanoë (1916) and is prevalent in the semi-arid bioclimatic stage and the regions bordering the dry region,¹ extremely common at altitudes between 800 and 900 m,³³ and its distribution extends eastward into Iran, Pakistan, and India. It is only found at a few stations in the Afro-tropical zone.³⁴ The species *P. sergenti* is less common than *S. minuta*, but it has a vast distribution area in the Fez region with high numbers, which could explain the existence of cases of leishmaniasis patients during this period because it is regarded as one of the species of medical interest. This result is consistent with the findings of Fellah *et al.* in the province of Zouagha Moulay Yaacoub,³⁶ and Talbi *et al.* in the Aichoun locality of Sefrou Province.⁶ Also, the results of Rioux *et al.* have demonstrated that this species is widespread across the Moroccan territory. Furthermore, the occurrence of this species has been demonstrated in different countries of the world, such as the Near East,³¹ and Ethiopia.³² In the province of Moulay Yaacoub, the results of Lahouiti *et al.*,^{13,14} revealed the dominance of another species, *P. papatasi*. The presence of *P. papatasi* was reported by Talbi in the Sefrou Province and by Lahouiti in the Moulay Yaacoub Province.^{6,13} The entire population of this species was recorded only at two sites. The presence of Zlilig and Dahrricha could explain the leishmaniasis cases reported from May 2017 to April 2018.

Phlebotomus perniciosus was ranked as the second among the species of the genus *Phlebotomus* with a relative abundance of 23%, and for all the specimens harvested (10.78%). This main vector species of VL is characterized by a wide geographical distribution from Portugal in the west to Crete and Turkey in the east and the countries of North Africa,³⁶ and occupies the whole of Morocco and reaches the Sahara.²²

Table 2: Results of catches during the study period

Species	Effective	M	F	Sex ratio	Density (ph/m ²)	R.Ab (%)	F.O (%)	Scale	RS
<i>Ph. sergenti</i>	220	212	8	26.5	14.69	26.96	58.3	C	
<i>Ph. papatasi</i>	74	72	2	36	4.94	9.07	41.67	A	
<i>Ph. perniciosus</i>	88	86	2	43	5.88	10.78	50	C	6
<i>S. minuta</i>	361	339	22	15.41	24.11	44.24	75	C	
<i>S. fallax</i>	65	62	3	20.6	4.34	7.97	50	C	
<i>S. antennata</i>	8	7	1	8	0.53	0.98	16.67	R	

RS: Specific wealth; M: male; F: female; R.Ab: Relative abundance; F.O: Frequency of occurrence; A: Accessory; R: rare; C: Common; VR: Very rare.



Figure 3: Spermatheca of *Phlebotomus papatasi* female (A) and genitalia of the male (B). (Original photo: G ×40)

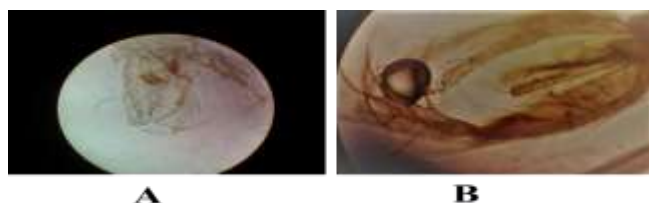


Figure 4: Genitalia of male *Phlebotomus sergenti* (A) and male *Phlebotomus perniciosus* (B). (Original photo: G ×40)



Figure 5: Genital organs of male *Sergentomyia minuta* (A) and spermatheca of *Sergentomyia munita* (B). (Original photo: G ×40)



Figure 6: Sandfly eggs. (Original photo: G ×40)

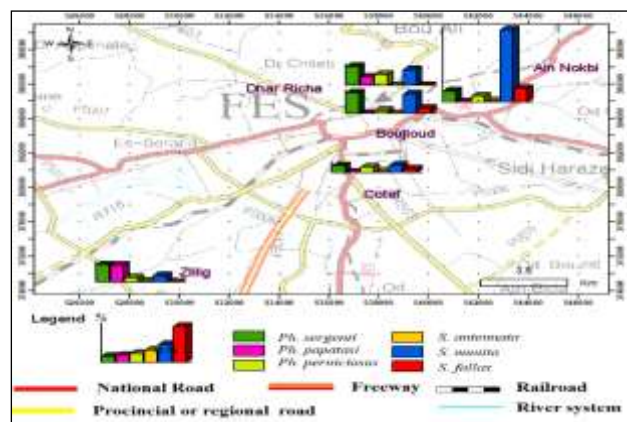


Figure 7: Spatial distribution of the sandflies species in different sites (Ain Nokbi, Boujloud, Coteff, Dhar Richa, and Zlilig) of Fez region, Central Morocco from May 2017 to April 2018.

In Algeria, its presence has been demonstrated by several studies, including that of Bounamous in 2010, because it is a zoo-anthropophilic species, which adapts well to the human and animal environment. Its existence in the station of Dhar Richa can be explained by the state of dilapidation of the houses with poorly maintained gardens. Also, the presence of the stables and the walls presents many anfractuosités favorable to the rest of the sandflies.^{35,36}

Frequency of occurrence of species

Based on the distribution of species, three groups can be identified, as depicted in Table 2. *Phlebotomus perniciosus*, *P. sergenti*, *S. fallax*, and *S. minuta*, are qualified as common, as they were found in more than 50% of the surveys carried out, while *P. papatasi* has a frequency of occurrence of 25-49% of the samples. Second, *S. antennata* is a rare species in the area, with a frequency of occurrence ranging from 10 to 25%.

Geographical analysis

To perform a geographical analysis of the above results, a sandfly distribution map was constructed using Qgis 2.18 software. The results obtained are shown in Figure 7. According to the GIS analysis of the geographical study, six species of sandflies were gathered and identified. *Phlebotomus sergenti*, *P. perniciosus*, and *S. munita* were found at the five sites in the Fez region, with a co-dominance of *P. sergenti* and *S. munita*. These two species are each in the lead at the level of trapping sites. *Sergentomyia minuta* was the most abundant species in the trapping stations in terms of effective representation. This species is dominant in the Ain Nokbi station and very rare in the Zlilig and Coteff stations due to their unfavorable ecosystems. In Algeria, the species that does not transmit the leishmania parasite, is the most abundant.²⁶ The distribution area of *P. papatasi* extends to Dhar Richa, and Zlilig, while *S. fallax* was mainly found in the site of Ain Nokbi but was also present in the sites of Coteff and Boujloud. *Sergentomyia antennata* species was absent in Dhar Richa and Boujloud and was found in the other sites, but much less frequently. According to Gebresilassie, knowledge of the distribution, population dynamics, and behavior of sandflies contributes to understanding the conditions for the acquisition of leishmaniasis infections.³⁷ The distribution of sandflies varies according to physicochemical conditions and soil type. More importantly, soil pH plays a vital role in the behavior of sandflies and the nature of the larval breeding habitat.^{38,18}

The results of the PCA revealed that the four stations (Ain Nokbi, Boujloud, Dhar Richa, and Zlilig) are likely biotopes with biological limiting factors for sandfly development, except for the Coteff station, which seems unsuitable for the development of these insects, where just a few specimens were found. Due to the presence of various types of trash, manure, and stables, it appears that the Dhar Richa, Ain Nokbi, and Zlilig stations are the most conducive biotopes for the survival of the sandfly population. Environmental factors such as temperature, humidity, and wind speeds, which are regarded as limiting factors for phlebotomy fauna, influence species distribution, and populations.

Conclusion

The specific richness, abundance, and spatial distribution of CL sandfly population vectors were the focus of this investigation. In general, the findings add to the understanding of the phlebotomian fauna of Fez Province, as well as possible CL vector species. This study found that sandfly development conditions are already present in Fez City, particularly during the vector activity period in May, posing a major health danger to the people of this prefecture. These findings necessitate further research into seasonal dynamics as well as an examination of the interaction between environmental and meteorological factors as well as the spatiotemporal distribution of sandfly vectors in Fez City.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

- Dedet JP. Leishmaniasis, leishmaniasis. biology, clinical and therapeutic. EMC. Elsevier (Masson SAS, Paris). Maladies infectieuses. 2009; 8:506-510.
- Gangneux JP, Belaz S, Robert-Gangneux F. Focus and news on Mediterranean visceral leishmaniasis. J Anti-Infect. 2015; 17(1):25-28.
- Rioux JA and Golvan Y. Epidemiology of leishmaniasis in the South of France. Monographie de l'I.N.S.E.R.M., Paris. 1969; 37:221-223.
- WHO. Continue to take action to reduce the global impact of neglected tropical diseases. WHO Second Report on Negl Trop Dis. 2010; 18p.
- Nasir S, Waleed MA, Arezki A. Leishmaniasis in the Middle East: Incidence and Epidemiology. PLOS Negl Trop Dis. 2014; 8(10):1-8.
- Talbi FZ, El Ouali Lalami A, Janati Idrissi A, Sebti F, Faraj C. Leishmaniasis in Central Morocco: Seasonal Fluctuations of Phlebotomine Sandfly in Aichoun Locality, from Sefrou Province. Pathol Res Int. 2015; Article ID 438749.
- Philippe D. Mediterranean bioclimate: Analysis of climatic forms by the Emberger system. JSTOR. 1977; 34(2):87-103.
- Dajoz R. Ecology Report. Ed. Bordas Paris. 1985; 505p.
- Zamrane Z. Research of indices of climate variability in hydroclimatic series in Morocco: identification, temporal positioning, trends and links with climatic fluctuations: the case of the large basins of Moulouya, Sebou, and Tensift. Earth Sciences. Montpellier University. Français. ffNNT : 2016MONTT181ff. 2016.
- Aouna K, Ben Abda I, Bousslimi N, Bettaieb J, Siala E, Ben Abdallah R, Benmously R, Bouratbine A. Comparative characterization of skin lesions observed in the three endemic varieties of cutaneous leishmaniasis in Tunisia. Annales de dermatologie et de vénéréologie. 2012; 139:452-458.
- Moroccan Ministry of Health. Department of Epidemiology and Disease Control, Leishmaniasis control, Activity Guide. 2010.
- WHO. Manual for the Management of Cutaneous Leishmaniasis in the WHO Region of the Eastern Mediterranean. 2014. available on: <https://int.ma/plus-de-3000-cas-de-leishmaniose-cutanee-maroc/>.
- Lahouiti K, El Ouali Lalami A, Hmamouch A, and Bekhti K. Phototropism of sandflies species (Diptera: Psychodidae) collected in a rural locality in Central Morocco. J Parasitol Vector Biol. 2014; 6(5):66-74.
- Lahouiti K, El Ouali Lalami A, Maniar S, and Bekhti K. Seasonal fluctuations of phlebotomine sand fly populations (Diptera: Psychodidae) in the Moulay Yacoub Province, Centre Morocco: effect of ecological factors. Afr J Environ Sci Technol. 2013; 7(11):1028-1036.
- Silvia RB, Uliana MD, PhD Jeronimo C. Ruiz PhD, and Angela K. Cruz. Leishmania Genomics: Where Do We Stand? In: Gruber A, Durham AM, Huynh C, et al., editors. Bioinformatics in Tropical Disease Research: A Practical and Case-Study Approach. Bethesda (MD): National Center for Biotechnology Information (US). 2008; Chapter B02. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK6821>.
- Bates PA. Transmission of Leishmania metacyclic promastigotes by phlebotomine sandflies. Int J Parasitol. 2007; 37:1097-106.
- Lane RP. Sandflies (Phlebotominae) In: Lane RP, Crosskey RW. (Eds). Medical Insects and Arachnids, Chapman and Hall, London. 1993; 78-119p.
- Talbi FZ, El Ouali Lalami A, Fadil M, Najy M, Ech-Chafay H, Lachhab M, Lotfi S, Nouayti N, Lahouiti K, Faraj C, Janati Idrissi A. Entomological Investigations, Seasonal Fluctuations and Impact of Bioclimate Factors of Phlebotomine Sandflies (Diptera: Psychodidae) of an Emerging Focus of Cutaneous Leishmaniasis in Aichoun, Central Morocco. J Parasitol Res. 2020; Article ID 6495108: 10p.
- El Omari H, Chahlaoui A, Talbi FZ, EL Mouhdi K, El Ouali Lalami A. Impact of Climatic Factors on the Seasonal Fluctuation of Leishmaniasis Vectors in Central Morocco (Meknes Prefecture). Canad J Infect Dis Med Microbiol. 2020; ArticleID 6098149: 7p.
- Talbi FZ, Faraj C, EL-Akhal F, El Khayyat F, Chenfour D, Janati Idrissi A, El Ouali Lalami A. Diversity and Dynamics of Sandflies (Diptera: Psychodidae) of Two Cutaneous Leishmaniasis Foci in the Fes-Boulemane Region of Northern Morocco. Int J Zool. 2015; Article ID 497169:6p.
- Rioux JA, Golvan YJ, Croset H, Houin R, Juminer B, Bain O, Tour S. Ecology of leishmaniasis in the South of France, Sampling, Ethology. Ann Hum Compar Parasitol. 1967; 42:561-603.
- Rioux JA, Lanotte G, Pratlong F. *Leishmania killickin*. sp. (Kinetoplastida: Trypanosomatidae), In: Leishmania Taxonomie et phylogénèse, Application éco-épidémiologique, Coll. Int. CNRS/INSERM. 1984, ed. IMEEE, Montpellier. 1986; 10665: 139-142.
- Rioux JA, Lanotte G, Retter F, Dereure J, Akalay O, Pratlong F, et al. Cutaneous leishmaniasis in the western Mediterranean basin. From enzymatic identification to eco-epidemiological analysis, the example of three foci, Tunisian, Moroccan, and French. Coll. Int. CNRS/INSERM. 1986; 365-395p.
- Boussaa S, Guernaoui S, Pesson B, Boumezzough A. Seasonal fluctuations of phlebotomine sand fly populations (Diptera: Psychodidae) in the urban area of Marrakech, Morocco. Acta Tropica. 2005; 95:86-91.
- Haddad N. The sandflies of Lebanon, Inventory and ecoepidemiological corollary. Faculty of Pharmacy of the University of Reims. 1998; 209p.
- Bounamous A. Biosystematic and Characterization by Molecular Biology of Phlebotomines of Eastern Algeria. Doctoral thesis. The University of Constantine. 2010; 304 p. Available from: <http://www.secheresse.info/spip.php?article80413>.
- Elaasri A, Belghyti D, Faraj C. Contribution to the study of sandflies in the Western region of Morocco: Notes on four species catch in Sidi Kacem Harrouch station. Science Lib, Editions Mersenne. ISSN. 2014; 6(141103):2111-4706.
- Al Zahrani MA, Peters W, Evans DA, Chin C, Smith V, Lane RP. *Phlebotomus sergenti*, a vector of Leishmania tropica in Saudi Arabia. Transac Roy Soc Trop Med Hyg. 1988; 82:416.
- Killick-Kendrick R, Rioux JA, Bailly M, Guy MW, Wilkes TJ, Guy FM, Davidson I, Knechtli R, Ward RD, Guilvard E, Perieres J & Dubois H. Ecology of leishmaniasis in the south of France. Dispersal of *Phlebotomus ariasi* Tonnoir, 1921 as a factor in the spread of visceral leishmaniasis in the Cévennes. Ann Hum Compar Parasitol. 1984; 59:555-572.
- Volf P, Ozbel Y, Akkafa F, Svobodova M, Votpyk J, Chang KP. Sandflies (Diptera: Phlebotominae) in Sanliurfa, Turkey: Relationship of *Phlebotomus sergenti* with the Epidemic of Anthroponotic Cutaneous Leishmaniasis. J Med Entomol. 2002; 39:12-15.
- Schnur LF, Nasereddin A, Eisenberger CL, Jaffe CL, El Fari M, Azmi K, et al. Multifarious characterization of

- leishmania tropica* from a Judean desert focus, exposing intraspecific diversity and incriminating *phlebotomus sergenti* as its vector. Am J Trop Med Hyg. 2004; 70(4):364-372.
32. Gebre-Michael T, Balkew M, Ali A, Ludovisi A, Gramiccia M. The isolation of *Leishmania tropica* and *L. aethiopica* from *Phlebotomus* (*Paraphlebotomus*) species (Diptera: Psychodidae) in the Awash Valley, northeastern Ethiopia. Transac Roy Soc Trop Med Hyg. 2004; 98:64-70.
 33. Rioux JA, Croset H, Léger N, Ben Mansour N, Cadi Soussi M. Presence of *Phlebotomus bergeroti*, *Phlebotomus chabaudi*, *Phlebotomus chadlii* and *Sergentomyia christophersi* in Morocco. Annales de Parasitologie Humaine et Comparee. 1975; 50:493-506.
 34. Abonnenc E. The sandflies of the Ethiopian region (Diptera: Phlebotomidae). ORSTOM dissertation. 1972; 55:1-289.
 35. Fellah H, Rhajaoui M, Ouahabi S, Belghiti D, Lyagoubi M. Occurrence of human cutaneous leishmaniasis in Zouagha My Yacoub Province (Morocco). Int J Agric Bio. 2007; 9(1):197-198.
 36. Mouloua A, Boubidi SC, Bouiba L, Mezai G, Madiou M, Harrat Z. Impact environnemental sur la répartition des leishmanioses dans le foyer de Tizi-Ouzou (Algérie). Revue de Médecine Vétérinaire, 2017; 168:252-261.
 37. Gebresilassie A, Kirstein OD, Yared S, Aklilu E, Moncaz A, Tekie H, Balkew M, Warburg A, Hailu A, Gebre-Michael T. Species composition of phlebotomine sandflies and bionomics of *Phlebotomus orientalis* (Diptera:Psychodidae) in an endemic focus of visceral leishmaniasis in Tahtay Adiyabo district, Northern Ethiopia. Parasites Vect, 2015; 8:248.
 38. Talbi FZ, Janati Idrissi A, Fadil M, El Ouali Lalami A. Soil analysis of potential breeding sites of sandflies (Diptera: Psychodidae) in Aichoun locality, central Morocco. Bull Soc Pathol Exotiq. 2020; 112(5):288-295.