**Edelweiss Applied Science and Technology**
*ISSN: 2576-8484*
*Vol. 8, No. 6, 9717-9726*
*2024*
*Publisher: Learning Gate*
*DOI: 10.55214/25768484.v8i6.4093*


# Epidemiology of cutaneous Leishmaniasis and taxonomy of phlebotomians sandflies in the Tafingoult village (Taroudant – Morocco)

EL GHRIFI Youssef[1], Abdelfatah BENCHAHID[1], BELGHYTI Nouhaila[1], Charaf FRAINE[1], Fatima Zahrae ELBAKRI[1,2], Riam TAIBA[1,3], Zakaria ELALAOUI[1,3], El Moctar HABIBY[1], Khadija ELKHARRIM[1], Driss BELGHYTI[1*]

[1]Laboratory of Renewable Resources, University Ibn Tofail, Faculty of Sciences Kenitra, Morocco; youselgh00@gmail.com (E.G.Y.) belghyti@hotmail.com (D.B.)
[2]Laboratory Med Biotech, University Mohamed V, Faculty of Medicine-Pharmacy, Rabat, Morocco.
[3]Health Center, Ain Dfali, Ministry of Health and Social Protection, Sidi Kacem, Morocco.

**Abstract:** Leishmaniasis is endemic in 66 countries in Southern Europe, Africa, the Middle East and Asia and in 22 countries in North, South and Central America. In Morocco, leishmaniasis was first described by Remlinger in 1913 and became a concern in the 1970s. The main objective of our study is to carry out a taxonomic inventory of sandfly vectors and to screen for cases of human leishmaniasis in Tafingoult village (Taroudant, Southern Morocco). Our work will strengthen the development of an eco-epidemiological research program for the eradication of leishmaniasis in this region. This collaborative survey was carried out between the Tafingoult Health Center and the Faculty of Sciences of Kenitra. The diagnosis of leishmanian wounds by the scraping method was carried out by a sterile vaccinostyle in the infiltrated part far from the superinfected areas. The product of the scraping and the collection of serosities were used to make stained smears with Giemsa and examined under a microscope with a 100 objective under immersion oil. The method of capturing sandflies focused on the aerial volatile phase using adhesive traps in the form of 25 x 20 cm surface paper panels impregnated with castor oil. The traps are placed overnight in the resting places of the sandflies. The next morning, the captured insects are removed and placed in labeled tubes containing 70% alcohol. Species identification is performed using guides or determination keys established by Bailly-Choumara and collaborators, based on morphoanatomical and biometric criteria. The results of the epidemiological survey revealed 87 positive cases of cutaneous leishmaniasis due to Leishmania *tropica* which were classified: 1 case had three lesions, 9 cases had 2 lesions and the rest of the 77 cases had a single lesion. The captured sandflies were identified as belonging to the species Phlebotomus *Sergenti* and Phlebotomus *longicuspis*. Our study revealed the persistence of cutaneous leishmaniasis in the region and suggests strengthening the epidemic eradication services.

***Keywords:*** *Epidemic, Health, Leishmaniasis, Parasites, Taroudant, Morocco, Vectors.*

## 1. Introduction

Leishmaniasis is a parasitosis caused by parasites of the Leishmania genus and transmitted by sandflies (Diptera; Family: Psychodidae) and which is listed among the five priority diseases of the WHO (Garcia-Stoeckel, 1992). It is an emerging disease and closely linked to the state of the environment (OMS, 1990).

The Leishmanian pathogenic complex (parasite, vector, reservoir) evolves in a geographical area defined by a set of bioclimatic parameters. Environmental changes have repercussions on the functioning of households, their dynamics, and their territorial extension. These modifications of the natural environment can be either of anthropogenic origin or of climatic origin; these two types of modifications are moreover most often closely intertwined.


* Correspondence: belghyti@hotmail.com

The emergence or re-emergence of leishmaniasis is directly linked to the increase in risk factors which are essentially anthropogenic in nature (Desjeux, 1999). Climate change contributes significantly to the increase and spread of vector-borne diseases, particularly leishmaniasis (Rodhain, 2000). Leishmania/HIV co-infections are an individual risk factor that favors the spread of the disease (Desjeux and Alvar, 2003).

Leishmaniases are endemic in 66 countries in the Old World (southern Europe, Africa, Middle East and Asia) and 22 in the New World (North, South and Central America) (Figure 1). The population at risk is estimated at 350 million people, with a prevalence of 12 million (Desjeux, 2004). Leishmaniases are increasing worldwide and currently, the estimate of the global annual incidence of leishmaniases cases is between 1.5 and 2 million, distributed between 1 to 1.5 million cases of cutaneous leishmaniases and the order of 500,000 cases of visceral leishmaniasis (WHO, 2000).

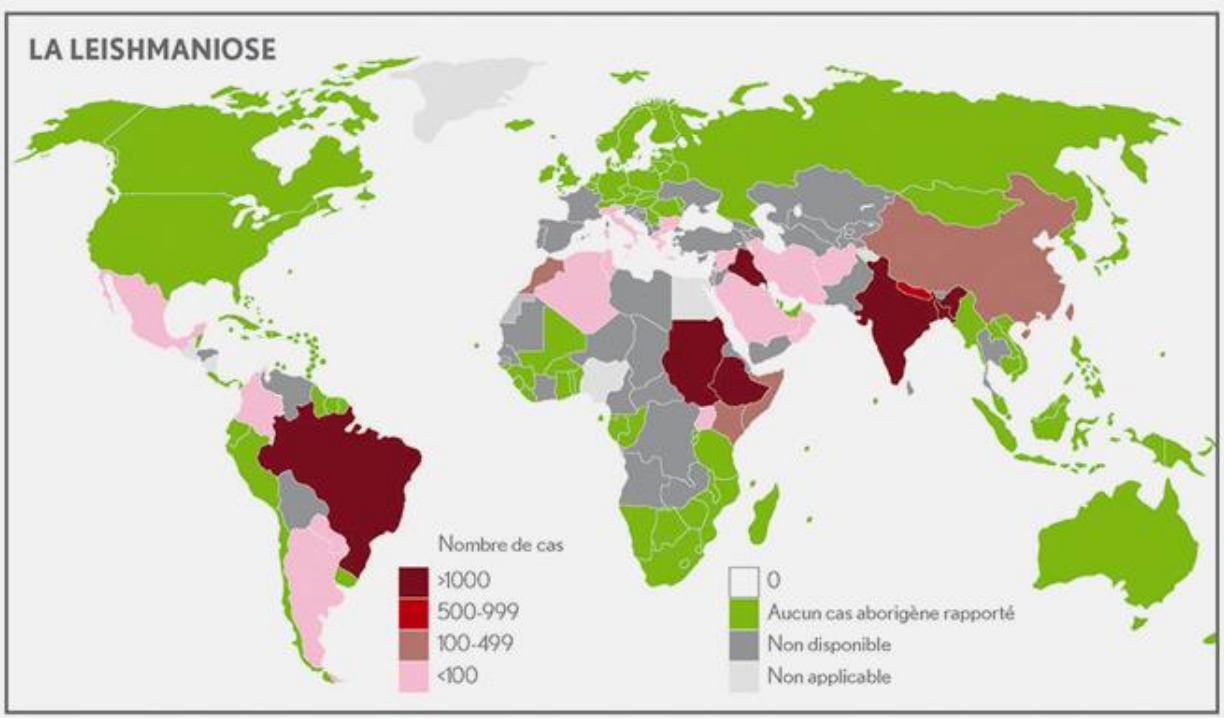


**Figure 1.**
Global distribution of leishmaniasis (WHO, 2000).

In Morocco, leishmaniasis was first described by Remlinger (1913) and became a concern in the 1970s, by developing an eco-epidemiological research program which made it possible to better understand the structure and dynamics of leishmaniasis cycles and to better study the sandflies of Morocco in an especially qualitative manner (Ristorcelli, 1947; Gaud, 1947; Bailly-Choumara, 1976; Dereure, 1991; Mahjour et al., 1992; Guessous Idrissi, 1997, 1999).

The vectors of leishmaniasis are sandflies (Diptera: Psychodidae); insects of the order Diptera. These insects play an important role in human pathology which for more than a hundred years has continued to arouse great interest due to their involvement proven on various occasions in the transmission of human and veterinary diseases. These sandflies can also transmit to humans the arboviruses responsible for the sandfly fever group and *Bartonella bacilliformis*, which causes Peruvian verruga and Oriya fever (Léger and Depaquit, 1999; 2001).

The region of Tafingoult (province of Taroudant) is not studied in depth with regard to the Leishmanian risk and the phlebotomian fauna, despite its position between the Leishmannian centers, namely the province of Elhaouz and Ouarzazate. So, a study of sandflies and leishmaniases in their natural environment is necessary for the establishment of a plan to combat leishmaniases in Tafingoult.

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 8, No. 6: 9717-9726, 2024*
*DOI: 10.55214/25768484.v8i6.4093*

Our study aims to characterize the epidemiological situation of leishmaniasis and populations of proven or potential leishmania vectors, in the study environment. This work is handled alternately between the Natural Resources Laboratory of the Faculty of Sciences of Kénitra and the health center of Tafingoult. It is part of research activities on the eco-epidemiology of leishmaniasis in the Tafingoult region.

The first part is devoted to the presentation of the results of the screening activity carried out at the Tafingoult health center, which outlines the clinical and epidemiological aspects.

The second part is based on the entomological study of the captured sandflies species, accompanied by interpretations by authors in this field, followed by a general conclusion closing our work.

## 2. Area, Materials and Methods of Study

*2.1. Study Zone*

The province of Taroudant was created on December 18, 1981 by Royal Decree No. 2-81-584 of Safar 1402 (12/18/1981). It is part of the Souss Massa Daraâ region (Figure 2) which is limited by:

- -The provinces of Chichaoua, Essaouira, and El Haouz in the North;
- -The provinces of Tata, and Tiznit in the South;
- -The province of Ouarzazate in the East;
- -The provinces of Inezgane Ait Mélloul, Agadir Ida Outanane, and Chtouka Ait Baha in the West.

The total area under a Tafingoult health district is 740 km$^2$. Dominant soils are Hamri. The majority of the surface area of the Tafingoult health district (almost 80% of surface area) is occupied, to the north, by the mountain ranges of the Grand Atlas which contain the sectors of Taouyalte, Chafarni and Tafingoult and the sector of Sidi Ouaziz which is located in a plain to the east. Water resources: 2 wadis and 15 springs.

The climate type is semi-arid; in summer dry and in winter cold. Average annual rainfall 150 mm / year. Average annual temperature is 25°C. Minimum annual temperature is 12°C. Maximum annual temperature is 43°C. Annual average wind is 25 km/h.

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 8, No. 6: 9717-9726, 2024*
*DOI: 10.55214/25768484.v8i6.4093*

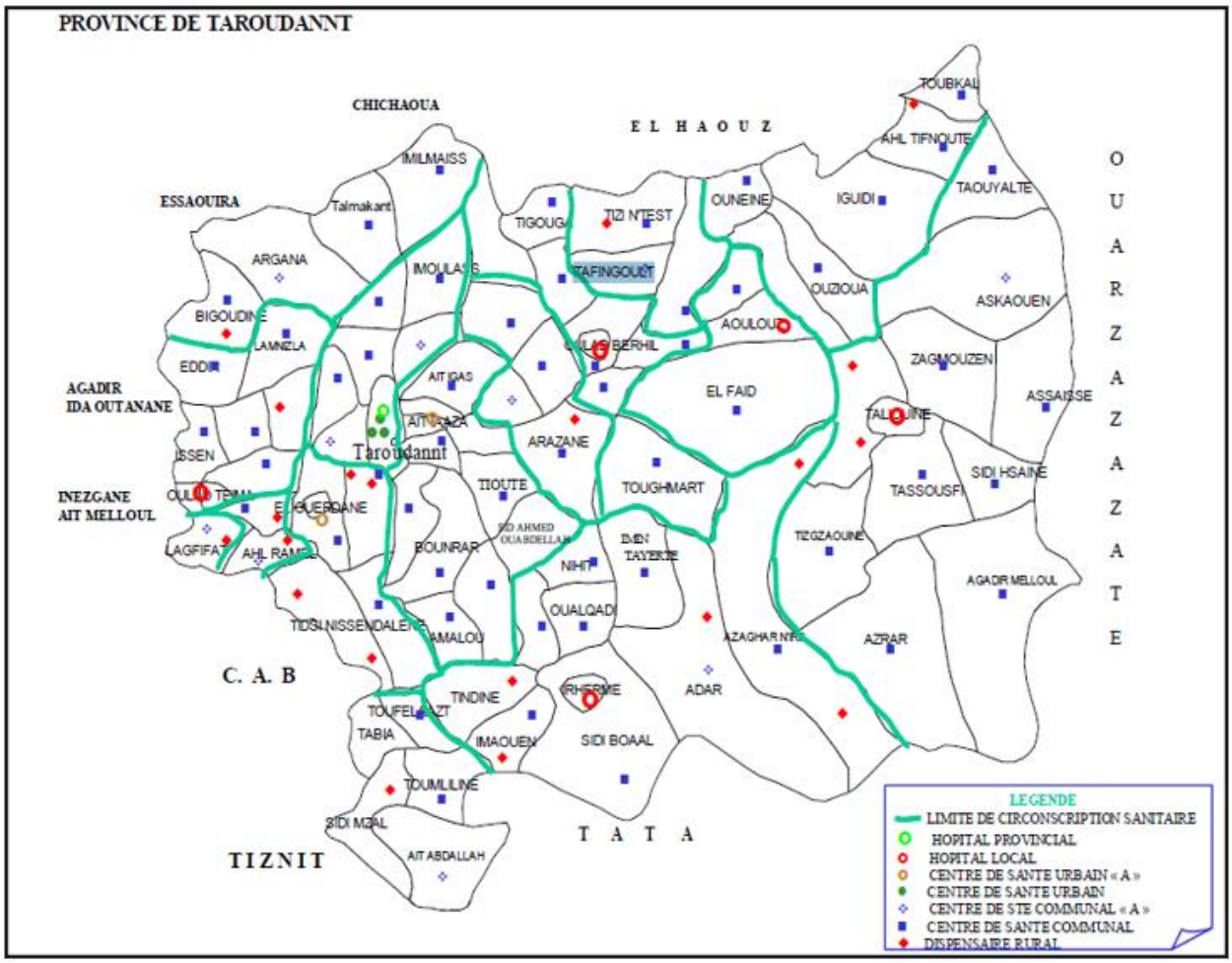


**Figure 2.**
Map of the boundaries of the health districts of Taroudant (Health Delegation, Taroudant, 2010).

*2.2. Clinical Diagnosis of Leishmaniasis*

There are two methods of collecting skin leishmaniasis (Figure 3):

**Scraping lesions**. The skin ulcer is carefully disinfected after removing the crusts covering it. Scraping is carried out by a sterile vaccinostyle in the infiltrated part far from the superinfected areas. The scraping product and the collection of serosities will be used to make the smears as well as for the production of cultures. Disinfection and covering the lesion with a protective dressing.

**Skin biopsy**. The biopsy is done using a punch. It allows for histological sections, slide appositions or cultures after crushing the sample in sterile physiological water containing antibiotics. Biopsy is indicated for non-ulcerated papulo-nodular lesions (El Alaoui et al.,2019).

Giemsa staining. The smears of bone marrow or skin serosities are fixed and stained with GIEMSA and this type of staining is carried out according to the following procedure and steps:

+Fix the spread with methyl alcohol for 2 to 5 minutes;
+Dry the blades;
+Color with 3% Giemsa in distilled water or buffered water at pH=7 for 15 to 20 minutes;
+Wash the slides with distilled water;
+Dry the slides in the oven;
+Examine under a microscope at 100 objective under immersion oil.

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 8, No. 6: 9717-9726, 2024*
*DOI: 10.55214/25768484.v8i6.4093*

*2.3. Sandflies Capture Technique*

It concerns the aerial phase of the development of the sandfly vector. Its execution, with standardized equipment and technique, enhances entomological evaluation.

Among various methods of collecting specimens of adult sandflies, procedures, requiring relatively simple equipment and technique, are proposed. The techniques for capturing sandflies are diverse and varied, but in our surveys, we have chosen the simple capture technique with adhesive traps because it is accessible and the means are available (Elassri et al.,).

Capturing sandflies with adhesive traps is the most used method in Morocco. We impregnate pieces of paper with a surface area of 25 x 20 cm, preferably 25 x 20 cm, with castor oil. Otherwise, we use A4 format paper (29.7 x 21) which we place overnight in the sandflies resting places (Houses, Stables, Henhouses, Schools, Gardens, ...)


**Figure 3.**
The stages of scraping the lesion (EL GHRIFI Youssef, Tafingoult Center, 2011).

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 8, No. 6: 9717-9726, 2024*
*DOI: 10.55214/25768484.v8i6.4093*

Depending on the shape of the trapped cavity, the traps are arranged either in a cone (cylindrical cavity) or vertically applied to a wall or fixed to a support (Figure 4). If necessary, they are held using a small flexible stick.

The papers are stripped as quickly as possible, to avoid deterioration of the captured sandflies, especially since the mushrooms develop quickly.

The sandflies are removed from the oil-impregnated paper using a fine brush soaked extensively in 75° alcohol and placed in tubes (per station) containing 75° alcohol with a label stating, in pencil, the date and station number. The capture results are expressed in number of sandflies per m² of adhesive paper per night.

**Figure 4.**
Adhesive traps for sandflies, installed in different biotopes at Tafingoult.

## 3. Results

### *3.1. Results of the Retrospective Study*

The general trend of the total number of people affected by cutaneous leishmaniasis from 1997 to 2008 appears irregular since there is an increase of 1669 cases in 2001, 1938 cases in 2007 and 1967 cases in 2008 (Figure 5).

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 8, No. 6: 9717-9726, 2024*
*DOI: 10.55214/25768484.v8i6.4093*

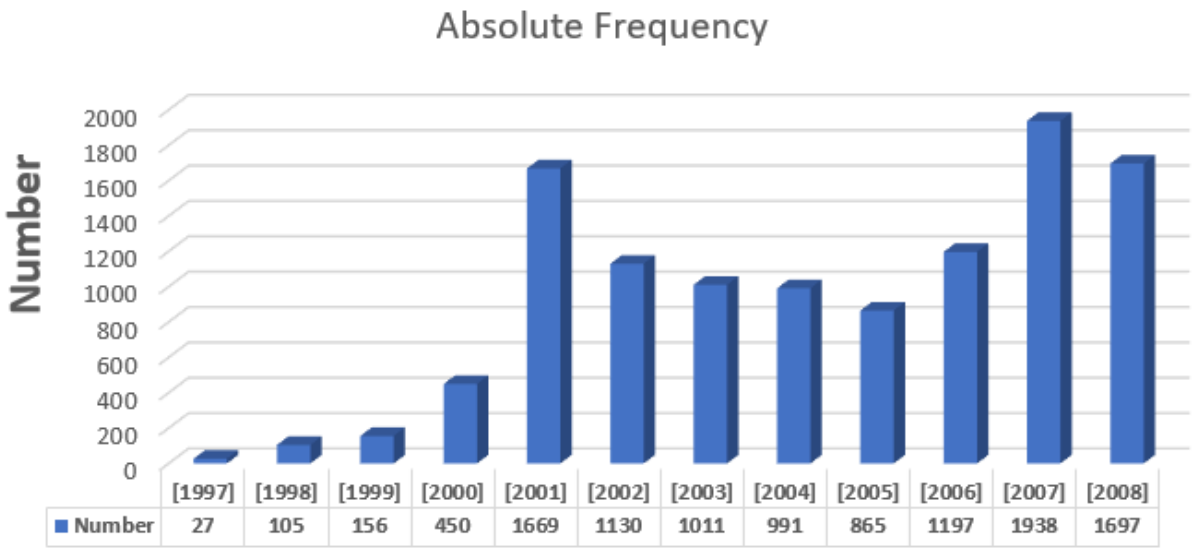


**Figure 5**.
Annual evolution of cases of cutaneous leishmaniasis due to Leishmania tropica, in the province of Taroudant (Parasitic Diseases Unit, Taroudant, 2010).

*3.2. Results of the Prospective Study*

Screening activity in the Tafingoult territory over a period of 5 years (from 2005 to 2010). During these 5 years, no cases of visceral leishmaniasis have been recorded in our territory. The results recorded concern cutaneous leishmaniasis caused by *Leishmania tropica*.

The results obtained reveal 87 positive cases which were classified:

- According to the clinical appearance (the site; the number and evolution of the lesion).
- According to the epidemiological aspect (sector, sex, age and type of screening).

Depending on the location of the lesions, almost all cases were affected in their faces, particularly under the eyes and only one case was affected in the hand. The face is most often uncovered when sleeping and therefore easily accessible to sandflies which bite at night.

According to the number of lesions, 1 case had three lesions, 9 cases had 2 lesions, and the rest of 77 cases had a single lesion. This is probably due to the rarity of biting sand flies or to the protection of residents against this sand fly.

According to the healing of the lesions, 3 cases had transferred lesions, 3 cases had lesions which evolved into scars and the other cases had complete healing of their lesions.

In 2005, the disease appeared in Chafarni with 5 indigenous cases and in two years following it, 2006 and 2007, the disease spread to Taouyalt and Tafingoult with an increase in the number of cases.

In 2008 the number of cases decreased then, in 2009, it will increase in Chafarni from 4 cases to 11 cases, and remain stable at the number of 6 cases in Taouyalt or reduce from 3 cases to 2 cases in Tafingoult.

In 2010, which remains an exceptional year in Taouyalt where the disease disappears while in Tafingoult and Chafarni the number of cases increases. The Sidi Ouaziz sector does not show any cases of leishmaniasis during this 5-year period.

*3.3. Results of Entomological Study*

The entomological study was undertaken in the 3 sectors of Tafingoult (Chafarni, Tafingoult and Sidi Ouaziz), by applying the trapping of 20, A4 format papers, impregnated with castor oil, in each sector and for a week. The 20 papers are placed in places where they allow effective capture of sandflies such as stables, waterways, trash areas, etc.).

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 8, No. 6: 9717-9726, 2024*
*DOI: 10.55214/25768484.v8i6.4093*

The sandflies captured by trapping site shows that the majority of sandflies collected are at the stables. This predominance at the level of the stables would explain the zoophilic nature of the insects in the sectors studied.

The capture results show us that most of the individuals are Culicoides and rarely individuals of sandflies (Figure 6). The captured sandflies belong to the genus Phlebotomus and are represented by two species: Subgenus Paraphlebotomus; species *Phlebotomus Sergenti* and Subgenus Larroussius; species *Phlebotomus longicuspis*.

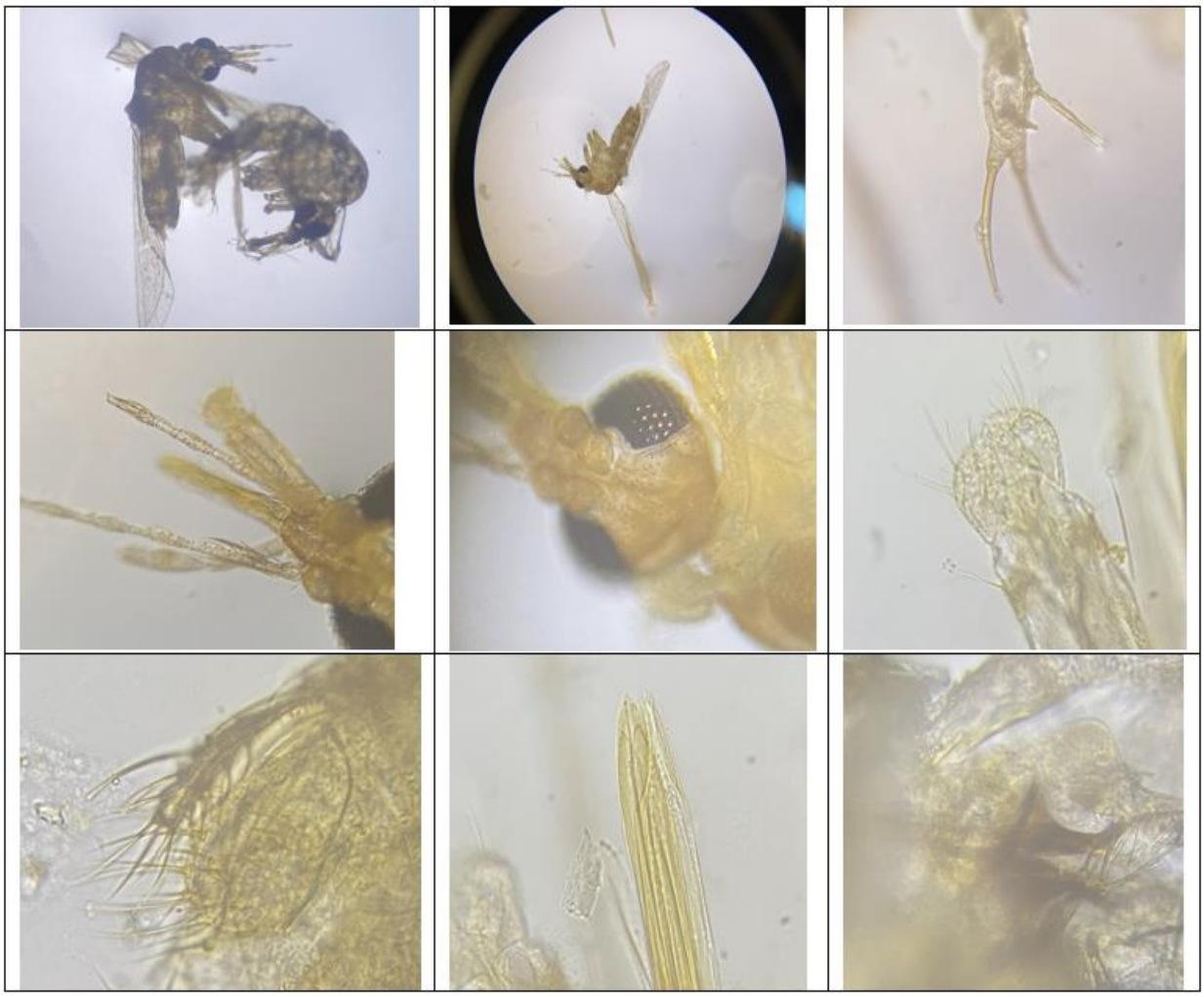

**Figure 6.**
Illustration of *Phlebotomus sergenti* under microscope (EL GHRIFI Youssef).

## 4. Discussion

This sequence of leishmaniasis from one sector to another may be due to the geographical location of the territory of Tafingoult and the watercourse which crosses the contaminated sectors since Chafarni (the first infected region) is located at the expense of the province of ElHaouz, also a focus of leishmaniasis and the source river in Chafarni which passes through Taoyalt and Tafingoult contributed to the infestation of these regions by cutaneous leishmaniasis.

The second reason which can explain this distribution of leishmaniases is that climatic changes and the sandfly-bioclimate correlations (Rioux et al., 1984) which are established with sufficient precision in the Old World.

*Edelweiss Applied Science and Technology*
*ISSN: 2576-8484*
*Vol. 8, No. 6: 9717-9726, 2024*
*DOI: 10.55214/25768484.v8i6.4093*

On the other hand, this difference is due to the nature of the environment and also its immediately surrounding environment. Thus, populations of *Phlebotomus Sergenti* find their optimum development in subhumid forests allowing *Leishmania tropica* to reach up to 1600 m altitude and be capable of triggering an active and stable outbreak of leishmaniasis.

This confirms our results because the areas studied are located in the High Atlas chain and which have subhumid forests except Sidi Ouaziz which is located in a plain and which has a semi-arid climate. Particularly, in terms of health, leishmaniasis appears to be an indicator of climate change (Rioux et al., 2001).

## 5. Conclusions

The epidemiological situation of leishmaniasis in Taroudant shows that in the region of Tafingoult where cutaneous leishmaniasis has become more widespread and acquired an epidemic character in rural areas with 87 cases during the period 2005-2010. However, regarding visceral leishmaniasis, no cases were recorded during this period.

We note the presence of *Phlebotomus Sergenti* in all sectors. It is a very important species from an epidemiological point of view. It exists throughout the Mediterranean basin where it is a vector of *Leishmania tropica*, the agent of anthroponotic cutaneous leishmaniasis (Guilvard et al., 1991).

In Morocco, it is found in many trapping stations distributed in varied geographical regions and in four bioclimatic stages (Rioux et al., 1986; Baily-Choumara et al., 1971; El Alaoui et al., 2016; 2019; El aasri et al., 2015).



## References

[1] Abonnec E. (1972). Sandflies of the Ethiopian region (Diptera: Phlebotomidae). ORSTOM Memoir. *55*, 1–289.

[2] Al Zahrani M.A., Peters W., Evans D.A., Chin C., Smith V. & Lane R.P. (1988). Phlebotomus sergenti, a vector of Leishmania tropica in Saudi Arabia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 82(3), 416-416. https://doi.org/10.1016/0035-9203(88)90142-3

[3] Anon., (2003). Status of programs to combat parasitic diseases. Directorate of Epidemiology and Disease Control. Annual Report of the Ministry of Health, Ministry of Health, Rabat, Morocco. 34–49.

[4] Bailly-Chaumara H., Abonnec E. & Pastre J. (1971). Contribution to the study of Moroccan sandflies (Diptera: Psychodidae). Faunal and ecological data. *Cah. ORSTOM, sér. Ent. Méd. Parasitol.* 9(4), 431-460.

[5] Guernaoui S. (2000). Contribution to the study of phlebotomes, potential vectors of leishmaniasis in the Marrakech region. DESA thesis, Cadi Ayyad University, Marrakech. 40p.

[6] Rioux, J.A. (1982). (Ed.), *Ecology of leishmaniasis. Paris: CNRS*. 139-151.

[7] Croset H., Rioux J-A., Léger N., Houin R., Cadi-Soussi M., Benmansour N., Maistre M., (1977). Sampling methods for sandfly populations in the Mediterranean region. Coll. Inter. CNRS, 239 " Ecology of leishmaniasis ", 139-151.

[8] Depaquit J., Léger N. & Killick-Kendrick R. (1998). Description de *Phlebotomus* (*Paraphlebotomus*) *riouxi* n. sp (Diptera-Psychodidae) d'Afrique du Nord. *Parasite*. 5, 151-158

[9] Depaquit J., Ferté H. & Léger N. (2000). Revision of the subgenus Paraphlebotomus (Phlebotomus Phlebotominae-Psychodidae-Diptera). Morphological and molecular approaches. *Ann. Pharm. Fr*. 58: 333-340.

[10] Dereure J., Velez I.D., Pratlong F., Denial M., Lardi M., Moreno G., Serres E., Lanotte G. & Rioux J.P. (1986). Indigenous visceral leishmaniasis in Southern Morocco. Presence of Leishmania infantum MON-1 in dogs in the pre-Saharan zone. Leishmania. Taxonomy and Phylogenesis. Eco-epidemiological applications. *Coll. Int. CNRS/INSERM*, 1984. IMEEE, Montpellier. 421- 425.

[11] Dereure J., Rioux J.P., Gallego M., Perieres J., Pratlong F., Mahjour J. & Saddiki A. (1991). *Leishmania tropica in Morocco: infection in dogs. Transactions of the Royal Society of Tropical Medicine and Hygiene*. 85, 595. https://doi.org/10.1016/0035-9203(91)90356-4

[12] Desjeux P. (1991). Information on the epidemiology of leishmaniasis and the fight against these diseases by country or territory. *OMS*., 30.

[13] Desjeux P. (1999). Leishmaniasis public health aspects and control. Edition Ellipses, 253p

[14] Desjeux P. & Alvar J. (2003). Leishmania /HIV co-infections: Epidemiology in Europe. *Annals of Tropical Medicine and Parasitology*. 97, 3-15. https://doi.org/10.1179/000349803225002499

[15] Desjeux P. (2004). Leishmaniasis: Current situation and new perspectives. *Comp Immunol Microbiol Infect Dis*. 27, 305-18. https://doi.org/10.1016/j.cimid.2004.03.004

[16] Elaasri A., Belghyti D., Laaroussi T., Hadji M, Grin. (2015a). Sand flies of Morocco: Biodiversity of the phlebotomienne fauna of Had Kourt region (Province of Sidi Kacem, Morocco). *Journal of Pharmaceutical, Chemical and Biological Science.* 3(2), 310-315
[17] EL Alaoui Zakaria, Amayour Abdelaziz, El Aasri Aziz, EL Kharim Khadija, Belghyti Driss, (2016). Cutaneous leishmaniasis in Ain Dfali, comparative epidemio-clinical aspects of 132 cases. European Scientific Journal January 2016 edition. 10.19044/esj. 2017.v13n12p60 ISSN: 1857 – 7881 (Print) e - ISSN 1857- 7431.
[18] EL Alaoui Zakaria, Amayour Abdelaziz, EL Kharim Khadija, Belghyti Driss, (2019). Cutaneous leishmaniasis in Ain Dfali province, west Morocco (2006-2015), International Journal of Civil Engineering & Technology (IJCIET) ISSN Print: 0976 - 6308 ISSN Online: 0976 – 6316 Paper ID: IJCIET/10/04/2019/IJCIET_43296.
[19] Flys-Sainte-Marie P.E. (1934). Premier cas de leishmaniose viscérale infantile observé dans la région de Fés. *Maroc méd.* 146, 320-332
[20] Gaud M. (1935). Visceral leishmaniasis in Morocco. *Bull. off. Int. Hyg. Publ.* 27, 533-535.
[21] Gaud J. (1947). Phlebotomes of Morocco. *Bull. Soc. Nat. Maroc.* 27, 207-212.
[22] Gaud J. (1954). Phlebotomes of Morocco. *Bull. Instit. Hyg.* Maroc. 14, 91-107.
[23] Gaud J. & Laurent J. (1952). Observations on sandflies in the Rabat region. *Bull. Soc. Sccs Nat. Maroc.* 27, 207-212.
[24] Guernaoui S., Boumezzough A. & Laamrani A. (2006). Altitudinal structuring of sand flies (Diptera: Psychodidae) in the High-Atlas mountains (Morocco) and its relation to the risk of leishmaniasis transmission. *Acta Trop.* 97, 346–351. https://doi.org/10.1016/j.actatropica.2006.01.001
[25] Guessous-Idrissi N., Chiheb S., Hamdani A., Riyad M., Bichichi M., Hamdani S. & Krimech A. (1997). Cutaneous leishmaniasis: an emerging epidemic focus of *Leishmania tropica* in north Morocco. *Trans. R. Soc. Trop. Med. Hyg.* 91, 660–663. https://doi.org/10.1016/s0035-9203(97)90511-3
[26] Guessous-Idrissi N. (1999). Cutaneous leishmaniasis in Taza. National Research, the Doctor's Notebooks. Tome II-N° 19, 37-40.
[27] Guilvard E., Rioux J.P., Gallego M., Pratlong F., Mahjour J., Martinezorlega E., Dereure J., Saddiki A. & Martini A. (1991). *Tropical Leishmania in Morocco III - Role of Phlebotomus sergenti.* A propos de 89 isolats1. *Ann. Parasitol. Hum. Comp.* 66, 96-99. https://doi.org/10.1051/parasite/199166396
[28] Léger N. & Depaquit J. (1999). Sandflies. *In Dedet J-P., Les Leishmanioses, Ellipses Ed.*, Paris, 89-108.
[29] Léger N., Depaquit J., (2001). Sandflies and their role in the transmission of leishmaniasis. French Laboratory Review 338, 41-48.
[30] Mahjour J., Akalay O. & Saddiki A. (1992). Leishmaniasis in Morocco from ecoepidemiological analysis to prevention. *DEPS Bul. Epidém.* Supp. N°7.
[31] OMS, (1990). Fight against leishmaniasis. *Geneva : Technical Report Series.* 176p.
[32] OMS, (2000). *Leishmania* and HIV co-infection. *Leprosy Review.* 71, 104-5.
[33] OMS, (2002). Urbanization: an increasing risk factor for leishmaniasis. *Weekly Epidemiological Record.* 77, 365-372.
[34] Rhajaoui M., Fellah H., Pratlong F., Dedet J. P. & Lyagoubi M. (2004). Leishmaniasis due to Leishmania tropica MON-102 in a new Moroccan focus. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 98**,** 299–301. https://doi.org/10.1016/s0035-9203(03)00071-3
[35] Rioux J.A., Rispail P., Lanotte G. & Lepart J. (1984). Phlebotomine-bioclimate relationships in leishmaniasis ecology. Epidemiological corollaries. L'exemple du Maroc. *Bull. Soc. Bot. Fr.* 131, 549–557.
[36] Rioux J.A., Lanotte G., Petter F., Dereure J., Akalay O., Pratlong F., Velez G., Fikri N.B., Maazoun R., Denial M., Jarry D.M., Zahaf A., Ashford R.W., Cadi Soussi M., Killick-Kendrick R., Benmansour N., Moreno G., Périères J., Guilvard E., Zribi M., Kennou M. F., Rispail P., Knechtli R. & Serres E. (1986a). Cutaneous leishmaniasis in the western Mediterranean basin: from enzymatic identification to eco-epidemiological analysis, the example of three 'foci', Tunisian, Moroccan and French. In Leishmania. *Taxonomy and Phylogeny. Eco-Epidemiological Applications, de. Rioux, J. A. Montpellier, France: Mediterranean Institute of Epidemiological and Ecological Studies.* pp. 365–395.
[37] Rioux J.A., Guilvard E., Dereure J., Lanotte G., Denial M., Pratlong F., Serres E. & Belmonte A. (1986b). Natural infestation of Phlebotomus papatasi (Scopoli, 1786) by Leishmania major MON-25. About 28 strains isolated in a focus in southern Morocco. In: *Leishmania. Taxonomy and Phylogenesis. Ecoepidemiological Applications. (Coll. Int. CNRS/INSERM, 1984), IMEEE, Montpellier, France,* 471–480.
[38] Rioux J.A. (2001). Thirty years of Franco-Moroccan cooperation on leishmaniasis: screening and analysis of outbreaks. Risk factors. Climate change and nosogeographic dynamics. *Pasteur Institute Alumni Association,* 168, 90–101.
[39] Ristorcelli A. (1940). On the sandflies of Morocco. *Pasteur Institute of Morocco.* 2, 367-381.
[40] Ristorcelli A. (1941). On the sandflies of Morocco (2° note). *Pasteur Institute of Morocco.* 2, 521- 533.
[41] Ristorcelli A. (1945). On the sandflies of Morocco (3° note). *Pasteur Institute of Morocco.* 3, 105- 109.
[42] Ristorcelli A. (1947). On the sandflies of Morocco (4° note). *Pasteur Institute of Morocco.* 3, 487- 488.
[43] Rodhain F. (2000). Health impacts: The case of vector-borne diseases. In: Potential impacts of climate change in France in the 21st century, Interministerial mission on the greenhouse effect. Paris: Ministry of Regional Planning and the Environment, 122-127.