

Sand flies (Diptera: Psychodidae) vectors of *Leishmania*: Notes on five species captured in Bni Oual locality, northwestern Morocco

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In order to assess the leishmanian risk related to the presence of sand flies in the Northwest of Morocco, a short entomological survey was realized in Bni Oual locality in May 2015. In total, 243 specimens of sand flies were collected. They belong to two genus, four Subgenus and five species. *Phlebotomus sergenti* is the dominant species of our capture with the proportion of 39.92% followed by *Phlebotomus longicuspis* (23.87%), *Sergentomyia minuta* (21,81%), *Phlebotomus papatasi* (14%) and *Phlebotomus ariasi* with one species identified.

Among these identified species, four of them are considered as vectors of *Leishmania* that causing cutaneous and visceral leishmaniasis which shows that the risk of vector-borne leishmaniasis is high in this Moroccan northwest locality.

Keywords : sand flies, vectors, leishmaniasis, Bni Oual , Morocco.

Afin d'évaluer le risque leishmanien lié à la présence de phlébotomes dans le Nord-ouest du Maroc, une petite surveillance entomologique a été réalisée dans la localité de Bni Oual en mai 2015. Au total, 243 spécimens de phlébotomes ont été collectés. Ils appartiennent à deux genres, quatre sous-genres et cinq espèces. *Phlebotomus sergenti* est l'espèce dominante de notre capture avec une proportion de 39,92% suivie de *Phlebotomus longicuspis* (23,87%), *Sergentomyia minuta* (21,81%), *Phlebotomus papatasi* (14%) et *Phlebotomus ariasi* avec une espèce identifiée. Parmi ces espèces identifiées, quatre d'entre elles sont des vecteurs confirmés de la *Leishmania*, agent causal de la leishmaniose cutanée et viscérale, ce qui montre que le risque de leishmaniose à transmission vectorielle est élevé dans cette localité du nord-ouest marocain.

Mots-clés: Phlébotomes, vecteurs, leishmanioses, Bni Oual, Maroc.

1 INTRODUCTION

Among the most important emerging and resurging vector borne protozoal diseases, the leishmaniasis are second only to malaria in terms of numbers of people affected (WHO, 2010). Sand flies (Diptera: Psychodidae: Phlebotominae) are the vectors of these major neglected tropical diseases worldwide (El Aasri *et al.*, 2016). Indeed, Among over 800 species of sandfly recorded, 98 are proven or suspected vectors of human leishmaniasis; these include 42 *Phlebotomus* species in the Old World and 56 *Lutzomyia* species in the New World (all: Diptera: Psychodidae) (WHO, 2010).

In Morocco, the list consists of 22 species of sand flies (Bailly-Chaumara *et al.*, 1971), distributed in 13 species of the genus *Phlebotomus* and 09 species of *Sergentomyia*. Only species of the genus *Phlebotomus* cause the transmission of leishmaniasis that is a real public health problem in this country (El Aasri *et al.*, 2015).

The overall objective of this investigation was to make a faunal inventory to specify the vector species of leishmaniasis in this locality which is part of the active Cutaneous leishmaniasis focus of Sidi Kacem province.

2 MATERIALS AND METHODS

2.1 Study Area and Environment

Our study was conducted at Bni Oual locality (34.72° N -5.65° W), a rural village in Sidi Kacem province, Northwestern Morocco. This village is 220 meters above sea level, with an annual mean temperature of 18.1°C and an annual average rainfall of 846 mm per year. It is characterized by the presence of two temporary ponds, cowsheds, and accumulation of animal waste that creates an environment favorable to the biological cycle of sand flies.

2.2 Sand fly sampling

The method used to capture sand flies is sticky-traps, particularly suitable for the qualitative and quantitative inventory of sand flies (Boukraa *et al.*, 2010; Kayedi *et al.*, 2016). These traps consist of white paper of standard A4 page size, coated with castor oil and held upright on wooden dowels. They were fixed at a height of 1 metre above ground level or placed at the entrance of rodent burrows and other crevices (holes in termite nests, soil cracks, tree trunks) in the evening around 6pm, and picked up until the next morning around 7am. In total, we have done performed a trapping of 29,3m² (473 traps) in 6 sites during the thirty successive days of Jun, 2015.

2.3 Sand fly identification

Specimens collected from each trap were stored in 70 % alcohol for morphological identification. They have been clarified by 10% potash for 4 hours and Marc-André liquid for 2 hours. Then they were mounted between slide and cover slip in the Marc Andre liquid (Hazratian *et al.*, 2016). The head is placed on its underside, its dorsal face in the upper position (for the *Phlebotomus* genus) to have an easy observation of pharyngeal armatures. Male genitalia and the distal portion of female abdomen are deposited on their side faces to observe the different elements necessary for this specific diagnosis.

Taxonomic identification is based on different morphological criteria described by Abonnec (1972), such as cibarium and pharynx in both male and female, spermathecae in female and the armature of genitalia in male (Abonnenc, 1972).

3 RESULTATS

3.1 Global inventory

The overall results of our short investigation in Bni Oual locality are shown in **Table 1**.

The entomological survey that we have conducted in the Bni Oual station allowed to capture 243 sandflies divided into two genus : *Phlebotomus* with three subgenus (*Phlebotomus*, *Paraphlebotomus* and *Larroussius*) and *Sergentomyia* with one subgenus (*Sergentomyia*). *Phlebotomus sergenti* is the most abundant species of our catches (39.92%) followed by *Phlebotomus longicuspis* (23.87%), *Sergentomyia minuta* (21.81%), *Phlebotomus papatasi* (14%) and *Phlebotomus arisai* with only 0.4% of the captured phlebotomines sand fly wildlife.

3.2 Distribution of species caught by sex

The results of the sex ratios of harvested species of sandflies are identified in **Table 2**.

In samples collected with oiled paper, a predominance of males to females was observed in four species (*Phlebotomus sergenti*, *Phlebotomus longicuspis*, *Phlebotomus papatasi* and *Phlebotomus arisai*). As against, female dominance was observed only in the species of *Sergentomyia minuta*.

Table 1: Number and abundance of sandflies species caught

genus	Subgenus	Species	number	Abundance(%)
<i>Phlebotomus</i>	<i>Paraphlebotomus</i>	<i>Phlebotomus sergenti</i>	97	39,92
	<i>Larroussius</i>	<i>Phlebotomus longicuspis</i>	58	23,87
		<i>Phlebotomus ariasi</i>	1	0,4
	<i>Phlebotomus</i>	<i>Phlebotomus papatasi</i>	34	14
<i>Sergentomya</i>	<i>Sergentomyia</i>	<i>Sergentomyia minuta</i>	53	21,81
Total			243	100

Table 2: species distribution by sex

Species	Male		Femele		Total	
	Nb	%	Nb	%	Nb	%
<i>Phlebotomus sergenti</i>	82	33,74	15	6,17	97	39,92
<i>Phlebotomus longicuspis</i>	37	15,22	21	8,64	58	23,87
<i>Phlebotomus papatasi</i>	30	12,34	4	1,65	34	14
<i>Phlebotomus ariasi</i>	1	0,41	0	0	1	0,4
<i>Sergentomyia minuta</i>	15	6,17	38	15,64	53	21,81
Total	165	67,90	78	32,1	243	100

4 DISCUSSION

Five species of sandflies, among the 22 listed so far in Morocco (Arroub *et al.*, 2012) were captured during our exploration in Bni Oual locality. Of these, two are implicated in transmission of cutaneous leishmaniasis, two others are confirmed vectors of visceral leishmaniasis and one species without epidemiological role.

Phlebotomus sergenti :

This species described in Algeria, occupies the entire Mediterranean area where it is incriminated in transmission of *Leishmania major* that causes anthroponotic cutaneous leishmaniasis (Ajaoud *et al.*, 2013). Also, recent studies have shown the incrimination of this sand fly in the transmission of *Leishmania killikii*, which in turn causes cutaneous leishmaniasis, particularly in Algeria and Tunisia (Boubidi, *et al.*, 2011). In Morocco, it is encountered in four bioclimatic with *Leishmania tropica* (Guernaoui *et al.*, 2009). Its identification based on characteristic ornamentation of coxite with basal Pedunculate lobe, carries a strong bristle brush (**Figure 1A**). On style, hail and cost, fit four thorns, the two distal being carried by subequal tubers. The female is equipped with spermathecae ringed four to six rings whose segments gradually decrease in height from the apex to the base (Fig1, B). Scaly beach occupying less than a quarter of the posterior pharynx and formed strong scales (Dantas-Torres *et al.*, 2014).

Phlebotomus longicuspis :

This species, vector of *Leishmania infantum* that insures visceral leishmaniasis is endemic in North Africa (Depaquit *et al.*, 2014) and ubiquitous in Morocco where it occupies the humid, semi arid and arid bioclimatic zones (Es-Sette *et al.*, 2014). The identification of the male of this species is essentially based on the morphology of penile sheaths: the valves are completed in a single tip, long, regularly tapered and slightly curved (**Figure 2A**). The female is characterized by its spermatheca ringed shuttle tank with six to nine segments and a long, thin collar. The conduit of this spermatheca is uniform from its origin to the



Figure 1: *Phlebotomus sergenti* ; **A-** Basal process of coxite; **B-** spermatheca of female

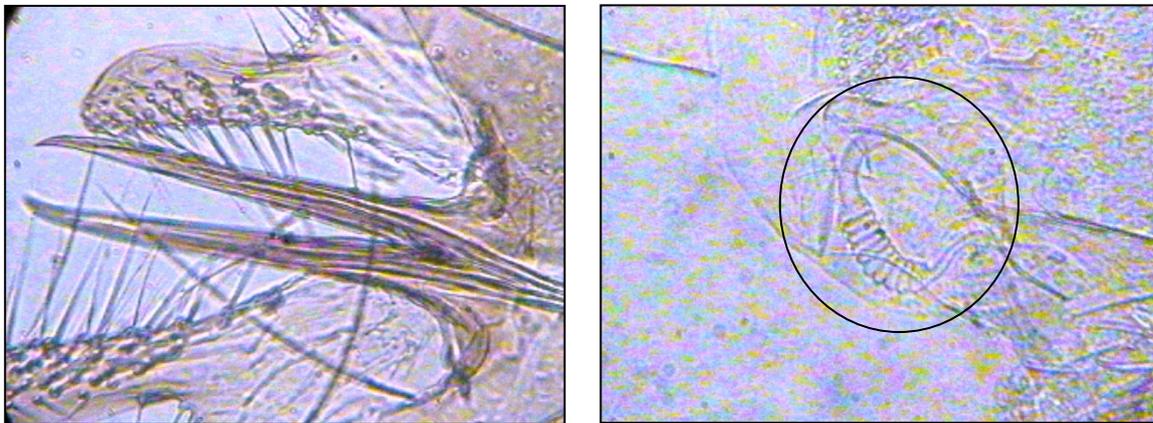


Figure 2: *Phlebotomus longicuspis*; **A :** Copulatory valves of male ; **B :** spermatheca of female

end (**Figure 2B**). The female is characterized also by its posterior pharynx, which has a homogeneous range.

***Phlebotomus papatasi* :**

This species described from Italy occupies the entire Mesogean influence area from Morocco to India (El Aasri *et al*, 2015). It is incriminated in the transmission of cutaneous leishmaniasis (Hanafi-Bojd, *et al*, 2016) and considered as one of the most common Mediterranean sandflies (Boussaa *et al*, 2016; Guernaoui, 2011). In Morocco, *Phlebotomus papatasi* is widespread in sub-humid, semi-arid, arid and Saharan bioclimatic zones (Rioux *et al*, 1997). The male of *Phlebotomus papatasi* primarily distinguished by the structure of styles that are long, narrow with 5 short and rigid spines, three implanted on the apical end, and two in the distal third of the lower edge (**Figure 3**). The female is characterized by its spermatheca with six to nine ring and without collar. The posterior pharynx contains in its posterior third scaly teeth whose shape and implantation are irregular ***Phlebotomus ariasi* :**

Species which describes the male from Spain and the female from France (Franco *et al*, 2010). It is ubiquitous in Morocco where it involved in the transmission of *Leishmania infantum* that causes visceral leishmaniasis (Rhajaoui, 2011; Kahime K *et al*, 2015). We have captured during our investigation only one male specimen. This last, is easily identified with the swelling of the penile valve (**Figure 4**).

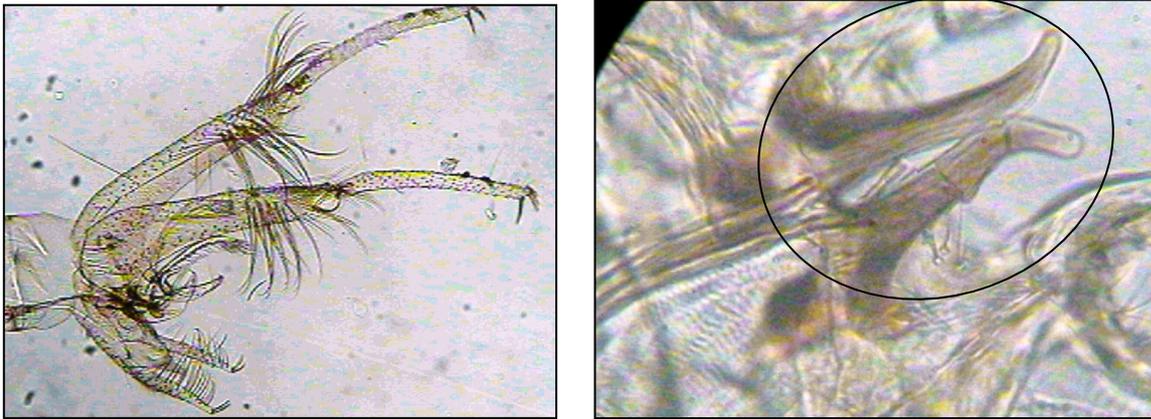


Figure 3: *Phlebotomus papatas*; **A** : Terminalia of male ; **B** : copulatory valves



Figure 4: *Phlebotomus ariasi*; copulatory valves

***Sergentomyia minuta* :**

Is a small sandfly described from North Africa including Morocco where it is abundant in the different bioclimatic from the humid to the Saharan (Kabbout *et al*, 2016). The male of this species which is not involved in the transmission of leishmaniasis (Boussaa *et al*, 2007) is characterized by its stocky style, equipped with 4 terminal spines and a non obsolete silk inserted to the union of proximal two thirds and the apical third (Figure 5A). While, the female has a completely smooth tubular spermatheca with undifferentiated reservoir (Figure 5B).

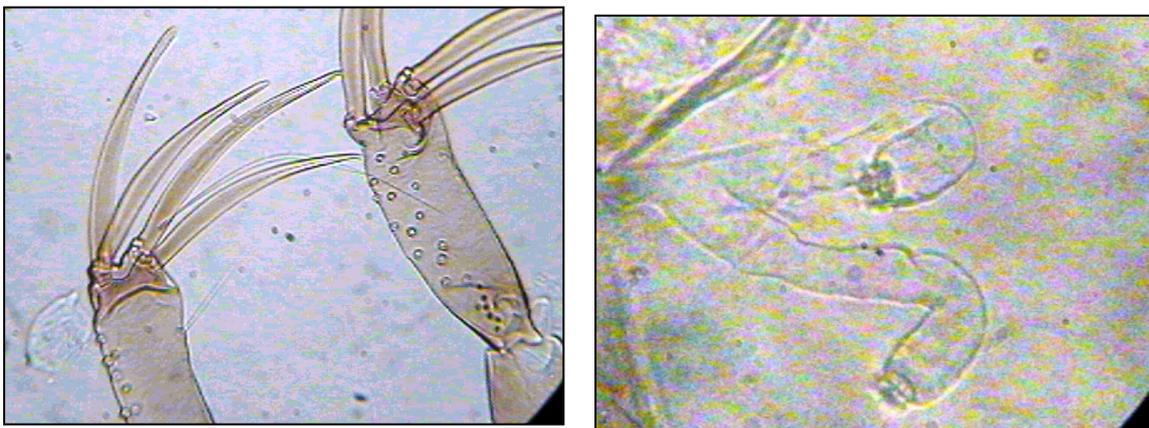


Figure 5 : *Sergentomyia minuta*; **A** : style of male ; **B** : spermatheca of female

5 CONCLUSION

This entomological prospection allowed the identification of five species of sandflies, which four are known to be vectors of *Leishmania*. These results confirm the presence of these insects potentially responsible of the transmission of *Leishmania* in Bni Oual locality part of the active focus of cutaneous leishmaniasis in Sidi Kacem province, northwestern Morocco.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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