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Diversity and occurrence of phlebotomine sand flies (Diptera: Psychodidae) in the area of Biskra (Middle Eastern of Algeria)

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Abstract
Phlebotomine sand flies are vectors of leishmaniasis. Both visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) exist in Algeria and are increasingly becoming a serious public health problem. This work aims to study the diversity and the occurrence of leishmania vectors in different parts of the region of Biskra. The study period was over a year with sampling, from May 2012 to April 2013 at 15 sites. The diversity of sand flies recorded showed the present ten species belong to two genera namely; *Phlebotomus* and *Sergentomyia.* The occurrence of the sand fly species revealed that *P. papatasi* and *S. minuta* were constantly present in all sites during the study period. *S. fallax* and *P. sergenti* were frequently occurring in the study areas. A moderate presence was observed for *P. bergeroti*, *P. perniciosus* and *P. alexandri* were recorded infrequently in the sites. *S. christophersi* and *P. chabaudi* were present sporadically in the study areas.

Keywords: Sand fly, phlebotomine, leishmaniasis, *Phlebotomus, Sergentomyia*

1. Introduction

Phlebotomine sand flies (Diptera: Psychodidae, subfamily Phlebotominae) are small-sized blood-sucking insects that feed on a wide range of hosts, and potentially transmit pathogens to man and other animals [1]. They are vectors of bacteria (e.g. *Bartonella bacilliformis*) [2], viruses (e.g. *Phlebovirus* and *Vesiculovirus*) [3] and protozoa (e.g. *Leishmania* spp) [4]. Among the estimated phlebotomine sand fly species, only 98 species of *Phlebotomus* and *Lutzomyia* are proven or suspected vectors of human leishmaniasis [5].

The genus *Leishmania* are protozoan parasites causing a spectrum of diseases called leishmaniasis, transmitted to humans through the bites of infected phlebotomine female sand flies. There are about 10 *Leishmania* species of significant importance for public health. Symptoms of leishmaniases can range from mild self-healing cutaneous lesions to fatal visceral cases [6]. Leishmaniasis is endemic to 98 countries or territories, in tropical and subtropical parts of the world. In Africa about 350 million people are at risk with about 12 million reported infections and about 60.000 deaths annually [7].

Phlebotomine sand flies are the main vectors known of both canine (L Can) and human (HL) leishmaniasis. These diseases are a serious and increasing public health problem in Algeria [1]. The HL is prevalent in 2 distinct forms; visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) in Algeria [8]. The vector of human (VL) is *Phlebotomus perniciosus* [9, 10, 11, 12]. The VL cases were firstly recorded in humid and sub-humid areas, between the Atlas chain and the coast in the northern part of Algeria [1, 13]. Since then, a resurgence of the number of cases and the appearance of new foci have occurred [1, 14, 15]. These zoonotic diseases were observed in 41 of 48 districts in Algeria [16]. CL calls attention by its quick extension from the old foci, thus becoming more frequently observed in the north of the country within the endemic foci of visceral *leishmania*. The CL is observed in 4 clinical forms caused by 4 different parasites. The first form, zoonotic cutaneous leishmaniasis (ZCL) of *Leishmania major* was identified in the steppe and Saharan regions and *Phlebotomus papatasi* was the vector [11, 17]. The second type, of the ZCL is caused by *Leishmania infantum*, is located in northern Algeria under sub-humid and semi-arid conditions [17] where it is transmitted by *Phlebotomus periciosus* [10] and the third form is of ZCL is due to *Leishmania killicki* identified in southern Algeria, at Ghardaia [15, 18] and in Annaba, at the extreme north-eastern part of Algeria by the Mediterranean sea [19] and other regions [20] but the sandfly vector has not been identified. The fourth form is *Leishmania tropica* which is transmitted from person-

to-person through *Phlebotomus (Paraphlebotomus) sergenti* in urban areas [21]. This old cutaneous disease (ZCL), also called 'Biskra boil', was formerly endemic in the sub-Saharan steppes (southern Saharan Atlas), where the most important foci are located. More recently, geographical spread of the disease towards the north and west (M'sila, Batna, Ksar Chellala, Djelfa and Bou-Saada) part of the country has taken place [16].

Monitoring vector communities is an integral part of disease surveillance and control programs. A marked increase in the number of cases of both VL and CL were reported at national scale. This infection increase could be explained by the extension of classical and the emergence of new foci on one hand and the presence and high abundance of the vectors across the country on the other hand. In order to provide information for the establishment of control and preventive programs against leishmaniasis, the present study was focused on the study of the diversity and the occurrence of sand fly species in the region of Biskra.

2. Materials and methods

2.1 Study area and sampling sites

The study area is in the district of Biskra (4°15' to 6°45'; 33°30' to 35°15'N) with an altitude ranging between 29 m to 1600 m above sea level and a surface of 21 671 km^2. This region, known as the door of the Sahara, is located in the middle-eastern part of Algeria situated in the southern part of the Saharan Atlas (Fig. 1). It is characterised by a Saharan climate, extremely hot and dry from May to September and humid and hot, from October to April. According to Bagnouls & Gaussen [22] the Ombrothermic diagram shows intensive dry seasons for all the study period (April 2012-May 2013) (Fig. 2).

In the present investigation, 15 different sites, varying between rural, suburban and urban type, were randomly selected to study the distribution of sand flies (Table 1).

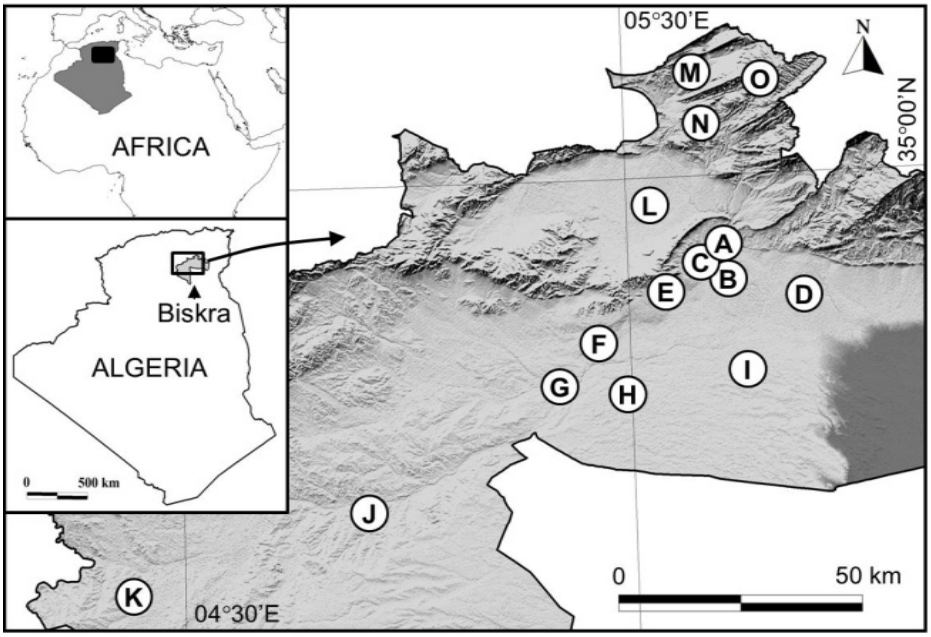

Fig 1: Geographic locations of the study sites in the region of Biskra (Letters present the sites listed in Table 1).

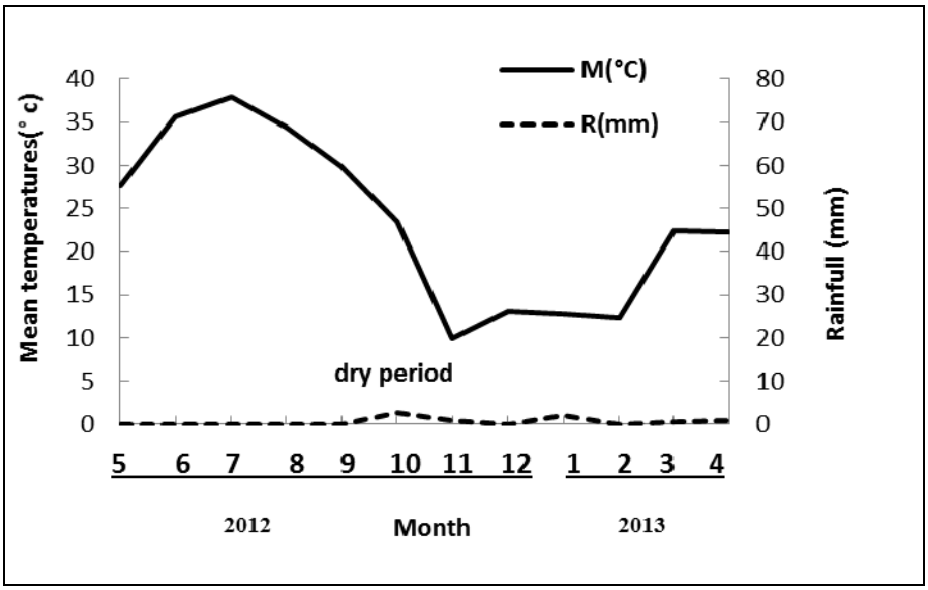

Fig 2: Gaussen Ombrothermic Diagram of the study period (May 2012-April 2013), of Biskra region.

Table 1: Geographic parameters of the study sites selected randomly in the Biskra region.

Site name	Code	Site type	Latitude	Longitude	Altitude
Alalia	A	Urban	34°51'5.20"N	5°44'5.79"E	112m
Feliache	B	Suburban	34°49'33.70"N	5°45'6.68"E	112m
Sidi-Ghzel	C	Suburban	34°49'18.08"N	5°41'49.50"E	112m
Sidi-Okba	D	Suburban	34°45'0.10"N	5°53'59.70"E	54m
Alhadjeb	E	Rural	34°47'30.25"N	5°36'4.03"E	146m
Bouchagroune	F	Rural	34°42'51.36"N	5°27'58.62"E	141m
Lioua	G	Rural	34°38'8.30"N	5°23'47.70"E	94m
Ourlal	H	Rural	34°38'22.71"N	5°24'16.52"E	83m
Oumache	I	Rural	34°41'26.12"N	5°42'13.18"E	40m
Sidi-khaled	J	Rural	34°22'60.00"N	4°58'60.00"E	207m
Raselmiaad	K	Rural	34°11'11.97"N	4°27'5.97"E	393m
Eloutaya	L	Suburban	35°1'60.00"N	5°35'60.00"E	253m
El-kantara	M	Suburban	35°13'31.00"N	5°42'23.00"E	519
Djemourah	N	Rural	35° 4'17.82"N	5°50'35.84"E	555m
Ain zaatout	O	Rural	35°10'13.94"N	5°51'21.80"E	831m

2.2 Collection of adult sandflies

Sand fly collection was carried out at each site, at irregular interval times during the study period and the sampling number was between 2 and 4 times per month. The adult sand flies were collected using CDC light-traps and adhesive paper placed in near habitations. The adhesive paper trap was made of a sheet white paper (Type A4) impregnated with Rici oil (Extract from *Ricinus communis* grains) [23]. The adhesive sheets were placed in different biotopes, domestics (house, courtyards, stable, and hen houses) and peri-domestics (garden, trees, walls or burrow of rodents). All the traps were collected in the following morning. Sand flies were separated from the other insects and preserved in alcohol at 70% in order to be identified. Specimens were subjected to a systematic study, by dissecting genital organs and following the morphological keys of phlebotomine sand flies of Algeria [24].

2.3 Data Analyses

The sand fly populations captured in the different sites of the study area were subjected to some analyses using ecological indices**.**

2.3.1 Pattern of occurrence (*C %*): To know the distribution of sand flies, the pattern of occurrence (C %) was estimated for all the study sites. Knowledge on the distribution pattern (*C %*) of phlebotomine sand flies reveals the dimension of spatial distribution in the selected study area. The pattern of occurrence of the phlebotomine species was analysed according to the method adopted by Rydzanicz & Lonc [25], when the following formula was applied: $C = n/N \times 100$. Where n = number of sites positive for the occurrence of sandflies and N = total number of sites studied. According to occurrence value sand fly species were classified into 5 categories:

If C = 0 – 20% the distribution pattern of the species is sporadic.

C= 20.1 – 40% the distribution pattern of the species is infrequent.

C= 40.1 – 60% the distribution pattern of the species is moderate.

C= 60.1 – 80% the distribution pattern of the species is frequent.

C= 80.1 – 100% the distribution pattern of the species is constant.

2.3.2 Relative Abundance (*RA %*): The dominance of the sand fly species for each site was estimated by the relative abundance (*RA %*). This was expressed by the ratio between number of specimens of a species and the total number of specimens of all sand fly species caught in the site ×100.

2.3.3 Specific Richness (*SR*): The specific richness of species was estimated by the total number of each species per site [26].

2.3.4 Equitability (*E*): The equitability was calculated in order to estimate the distribution and the organisation of sand fly population in the study site, following the present formula ($E = (IS-1)/(SR-1)$). E varies from 0 (dominance of a species) to 1 (all species populations equitably distributed) [26]. The specific biodiversity of study sites was evaluated by the Index of Simpson's diversity (*IS*). This reveals the relationship between the number of species and the number of specimens at the same time. It was calculated according to the following formula: $IS = 1/(\Sigma\ Pi2)$. Pi is the proportion (P) of the species (i) in a study site (Pi= RA/100). In order to confirm the hypothesis that there is no difference in the density of collected sand flies and diversity indices (*SR, IS, E*), between the 15 study sampled sites, Pearson's Chi-square test ($\chi 2$) was carried out at the limit of $P<0.05$.

3. Results

In the present investigation, the distribution of phlebotomine fauna of Biskra region is studied and prevalence throughout the period of study is analysed. The diversity of sand flies recorded in the study area showed the presence of 10 species, belonging to 2 genera; *Phlebotomus* and *Sergentomyia* (Table 2). A total of 1458 adult of phlebotomine sand flies were collected from all study sites during the study period. Results of the occurrence analysis showed that *Phlebotomus papatasi* (100%) and *Sergentomyia minuta* (93.33%) were constantly present during the study period (Table 3). According to the values of C% which varied between 66.67 and 80.00%, *Sergentomyia fallax*, and *Phlebotomus sergenti* were found to be occurred frequently in the study areas. A moderate occurrence (C= 46.67%) of species was observed only for *Phlebotomus bergeroti* with in the sites. The C% of *Phlebotomus perniciosus* (40%) and *Phlebotomus alexandri* (33.33%) revealed that their occurrence was infrequent. S*ergentomyia christophers* and *Phlebotomus chaboudi* (13.33-20%) occurred sporadically in the study areas during the study period. The pattern of occurrence showed that the cutaneous leishmaniasis vectors, *Phlebotomus papatasi* and *Sergentomyia minuta,* are the species that were distributed predominantly in the study area during the study period.

Table 2: Sand fly species identified in the study sites, during the study period (May 2012–April 2013).

S. n°	Name of the Species
1	*Phlebotomus papatasi* (Scopoli, 1786)
2	*Phlebotomus sergenti* (Parrot, 1917)
3	*Phlebotomus bergeroti* (Parrot, 1934)
4	*Phlebotomus perniciosus* (Newstead, 1911)
5	*Phlebotomus alexandri* (Sinton, 1928)
6	*Phlebotomus chaboudi* (Croset, Abonnenc & Rioux, 1970)
7	*Sergentomyia minuta* (Adler &Theodor, 1927)
8	*Sergentomyia fallax* (Parrot, 1921)
9	*Sergentomyia antennata* (Newstead, 1912)
10	*Sergentomyia christophersi* (Sinton, 1927)

Table 3: The pattern of occurrence of phlebotomine sand fly species sampled during the study period (May 2012-April 2013).

Name of the Species	*C* (%)	Occurrencepattern	Species number
Phlebotomus papatasi	100	Constant	2 species
Sergentomyia minuta	93.33	Constant	
Sergentomyia fallax	80	Frequent	3 species
Sergentomyia antennata	73.33	Frequent	
Phlebotomus sergenti	66.67	Frequent	
Phlebotomus bergeroti	46.67	moderate	1 specie
Phlebotomus perniciosus	40	infrequent	2 species
Phlebotomus alexandri	33.33	infrequent	
Sergentomyia christophersi	20	sporadic	2 species
Phlebotomus chaboudi	13.33	sporadic	

The study was carried out during 2 distinct periods, hot dry period and humid hot period. The investigation revealed the presence of different species where the number varies from 5 to 9 species in the sites and these were predominantly distributed during the hot dry period (May - October 2012). Whereas during the period, ranging from December to March, the presence of sand flies were completely absent. During the survey the emergences of some of these species appear again in April and the presence of 5 sand flies species were recorded (Table 4). We noticed that, from the same results 6 different species, *S. minuta, S. fallax, S. antennata*, *S. christophersi*, *P. sergenti* and *P. papatasi* were present in the all sites during 8 successive months, from April to November. The diversity indexes (IS) of sand flies species showed an evolution, more the specific richness (SR) increases the diversity progresses (Table 5). These both indexes are high practically in all study sites, with specific richness (*RS*) varies between 6 and 10 for the majority of sites. The distribution of the species was estimated by the equitability test and the results showed a moderate presence of species with values of *E;* varies between 0.4 and 0.8 that signifying a dominance of some species according to others which represent a low dominance. For the sites A, D and K the *SR* = 2 traduces a weak and non stable mediumof breading species. The Chi-squared test revealed no differences (P>0.05) between the study sites for values of all parameters of diversity (*SR, IS, E*).

Table 4: Temporal distribution of the phlebotomine sand fly species sampled in the study sites, during the study period (May 2012-April 2013).

Species Name	Temporal distribution											
	Very hot and dry period						Soft and dry period					
	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
S. minuta	+	+	+	+	+	+	+	-	-	-	-	+
S. fallax	+	+	+	+	+	+	+	-	-	-	-	+
S. antennata	+	+	+	+	+	-	-	-	-	-	-	-
S. christophersi	+	+	+	-	+	-	-	-	-	-	-	-
P. sergenti	+	+	+	+	+	+	+	-	-	-	-	+
P. perniciosus	-	-	+	+	+	+	-	-	-	-	-	-
P. papatasi	+	+	+	+	+	+	+	-	-	-	-	+
P. chabaudi	-	-	+	+	+	-	-	-	-	-	-	-
P. bergoroti	-	+	+	+	+	-	-	-	-	-	-	-
P. alexaondri	+	+	-	-	-	-	-	-	-	-	-	+
Total number of species	7	8	9	8	9	5	4	0	0	0	0	5

Table 5: Spatial distribution of diversity indexes and the dominance of phlebotomine sand fly species sampled in the study sites during the study period (May 2012-April 2013). (*SR*: Specific Richness, *IS*: Index of Simpson, *E*: Equitability, RA: Relative Abundance (%).

Site code	*SR*	*IS*	*E*	Dominant species
A	2	1.80	0.80	*S. minuta* (66.7), *P. papatasi* (33.33)
B	10	4.60	0.40	*P. papatasi* (29.4), *S. minuta* (23.75), *S. fallax* (21.25)
C	8	2.54	0.22	*P. papatasi* (60.2)
D	2	1.47	0.47	*P. papatasi* (80), *S. fallax* (20)
E	6	2.70	0.34	*P. papatasi* (54.55), *S. antennata* (24.24)
F	7	4.43	0.57	*P. papatasi* (32.5), *S. fallax* (22.5)
G	8	3.93	0.42	*P. papatasi* (36.22), *S. minuta* (26.44)
H	6	3.33	0.47	*P. papatasi* (44.83), *S. minuta* (22.76)
I	9	4.16	0.39	*P. papatasi* (35), *S. fallax* (23.5)
J	10	5.74	0.47	*P. papatasi* (32.56)
K	2	02.00	1	*S. minuta* (50), *P. papatasi* (50)
L	6	4.17	0.63	*P. papatasi* (31.82), *S. antennata* (27.3), *S. minuta* (22.73)
M	10	6.12	0.57	*P. papatasi* (27.6), *S. minuta* (18.9)
N	7	04.48	0.58	*P. papatasi* (36.36), *S. fallax* (18.2), *P. perniciosus* (18.2)
O	6	3.77	0.55	*S. minuta* (43.24), *P. papatasi* (18.92)
χ^2_{15}	17.60	7.75	9.56	
P	0.226	0.902	0.846	

4. Discussion

Diversity study and geographic distribution of vector-borne diseases are relevant for understanding ways of parasite transmission [27]. In a complex endemic leishmaniasis with different clinical manifestations, the diversity of sand fly vector species and mammal reservoir hosts may differ depending on the geographic region [28]. Phlebotomine sand flies are present in ecological settings that vary from very humid tropical forest to deserts, from temperate cities situated at sea level to high mountain villages. Despite this diversity all sand fly species share a number of basic features. All are nocturnal, hiding during the day in dark, humid microhabitats and able to insert themselves into confined spaces to avoid extremes of temperature or humidity.

The present entomological study in Biskra region showed the presence of 10 sand fly species which are belonging to 2 genera, *Phlebotomus* and *Sergentomyia*. The first genus is represented by the 6 species; *P. papatasi, P. sergenti, P. bergeroti, P. perniciosus, P. alexandri* and *P. chabaudi*. The *Sergentomyia* genus is represented by S. *minuta, S. fallax, S. antennata* and *S. christophersi*. The sand fly diversity is characterised by the dominance of 2 species, *P. papatasi* and *S. minuta* followed by *S. fallax, S. antennata* and *P. sergenti.*

The seasonality of the sand fly fauna in Biskra region was examined and result showed it to be active only during the dry hot season that ranging from April to November. The *Phlebotomus perniciosus* vector of human VL was recorded only in one site with a dominance of 18.2%. However *Phlebotomus papatasi,* the main vector of CL major, was identified in the different study sites with the highest abundance of sand fly species.

The exploration of the localities in the region of Biskra revealed a low diversity according to the 22 known species of Algeria. 6 species only are dominant, with a pattern of occurrence (C %) varies between 46.67%; that was considered moderate, and 100% that was constant. The 4 other species; *P. perniciosus, P. alexandri, P. chabaudi* and *S. christophersi* there distribution was inconstant and there pattern of occurrence was infrequent or sporadic. In a northern region of Algeria only 5 species were identified in Oum El Bouaghi district [16] and 4 species in Constantine [29] and Mila [30]. In north-eastern, by the Mediterranean Sea (Skikda), the presence of 5 species was reported [31]. A closer similar diversity of sand fly species was represented during the morphotaxonomic study in the region of M'Zab-Ghardaia [32]. This survey showed the presence of 7 species from the same 2 genera, *Phlebotomus* and *Sergentomyia* and the most abundant species were respectively *S. antennata*, *P. papatasi*, *S. minuta* and *S. fallax* [32].

The distribution and the density of sand fly species were inconstant, because of during the sampling some species were present with large number of adults while others were found present only with a few ones. Therefore, the difference in the sand fly density in the different study sites was probably due to the phenology of species during the study period [16] and the biotopes and climatic conditions, including temperature, precipitation and wind speed, which play the role of limiting factor for many flying insects [33].

5. Conclusion

The survey of the temporal and spatial distribution of sand fly communities; when leishmania disease transmission is likely occurred, become an integral part for drawing out surveillance programs and control measures. The diversity study and the establishment of the general distribution of sand fly species are directly implicated in the spread of infectious leishmania diseases (VL and CL) and are related to local transmission dynamics. While this result contributes to the existing survey of sand fly vectors in Biskra region, it is important to consider that seasonal fluctuations of phlebotomine insects which are varied across the year, affecting both the abundance of individual species and the diversity composition, and their subsequent response to climatic variation.

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