Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 2820-2826

Received: 10-01-2018
Accepted: 11-02-2018

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Study on the distribution of phlebotomine (Diptera, Psychodidae) responsible for human leishmaniasis based on biotopein type from the region of Constantine, Algeria

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Abstract
The present investigation took place in the region of Constantine in northeast of Algeria, during the period from April 2013 to October 2013 and from April 2014 to October 2014; she was conducted to study the biodiversity and chorology of Phlebotomian fauna in this region. For the sampling process, we adopted the technique of the adhesive traps deposited in various stations, where we explored internal biotopes (farms) and external ones (retaining walls). The results of the morpho-taxonomic study on the collected sand flies showed the presence of both types of sand flies: *Sergentomyia* and *Phlebotomus*. The identified species were 5 in the internal biotopes and 4 in the externalbiotopes. These are *Sergentomyia minuta parroti* Rondani 1843 (53.16%), *Phlebotomus perniciosus* Newstead 1911 (43.46%), *Phlebotomus longicuspis Nitzulescu* 1930 (2.84%), *Phlebotomus perfiliewi* Parrot 1930 (0.37%) and *Phlebotomus papatasi* Scopoli, 1786 (0.18%) identified in the internal biotopes. While in the external biotopes, the species identified were: *Sergentomyia minuta parroti* Rondani 1843 (51.01%), *Phlebotomus perniciosus* Newstead 1911 (39.60%), *Phlebotomus longicuspis* Nitzulescu 1930 (6.94%), *Phlebotomus perfiliewi* Parrot 1930 (2.46%). This inventory shows the presence of two species of subgenus *Larroussius* (*Phlebotomus perfiliewi* and *Phlebotomus perniciosus*); proven vectors of *Leishmania infantum* responsible for visceral leishmaniasis. The species *Phlebotomus papatasi*, vector of *Leishmania major* responsible for cutaneous leishmaniasis is also present.

Keywords: Phlebotomine, inventory, Constantine, Leishmaniasis

Introduction
Leishmaniasis (an environmental disease) [1] is reported throughout the country [2]. Their known vectors are phlebotomines, which is also responsible for the transmission of certain diseases [3, 4]. Algeria is amongst the countries most affected by leishmaniasis, which turns out to be a real public health issue. The disease is endemic in three clinical forms: visceral leishmaniasis (VL), sporadic cutaneous leishmaniasis (SCL) found in the north and zoonotic cutaneous leishmaniasis (ZCL) [5].
Visceral leishmaniasis (VL) is very abundant in the north of the country [6]. It is mostly present in the humid and sub-humid stage but is also found in the arid and semi-arid soils (M'sila, Batna, Biskra). However, the most affected areas are Jijel, Mila, Constantine, and Skikda.This lesion of visceral leishmaniasis is caused mainly by *Leismania infantum* whose known source is the dog [7], and the main vector is *Phlebotomus perniciosus* [8].
Algeria is a fertile ground for the spread of two forms of cutaneous and visceral diseases due to its large rural population and its different bioclimatic stages; ranging from the Mediterranean climate in the North, sub-humid and semi-arid zones to the Saharan climate in the South [9, 10, 11]. To better understand the diversity of sand flies in the region of Constantine and to make a comparison between the biotopes (internal and external ones), the present study was intend to establish initially an inventory of Phlebotomine from the stations prospected in this study for a better appreciation of the information collected from the University Hospital of Constantine regarding the cases of visceral and cutaneous leishmaniasis.

2. Material and methods
2.1 The Study Area
The present entomological survey lasted 14 months during the period from April 2013 to October 2013 and from April 2014 to October 2014.

These periods correspond to the activity of sandflies. The sampling took place in Constantine located eastern Algeria (36° 36'N, 06° 62 E, 660 m). It covers an area of 2287 km² and extends over a rocky plateau at 649 meters above sea level. It is limited on the north by the state of Skikda, on the west by the state of Mila, on the east by the state of Guelma and on the south by the state of Oum-El-Bouaghi. The state of Constantine enjoys a Mediterranean climate and is subject to a semi-arid climate with cool winters.

This study was carried out in four places located in the center and on the suburbs of the city of Constantine; these suburbs are Didouche Mourad (DM), Zighoud Youcef (ZY) and Beni H'midene (BH), located to the north of the city towards the state of Skikda, and Chaabet Erssas (CR) station located downtown. Seventeen sites were surveyed during the present investigation.

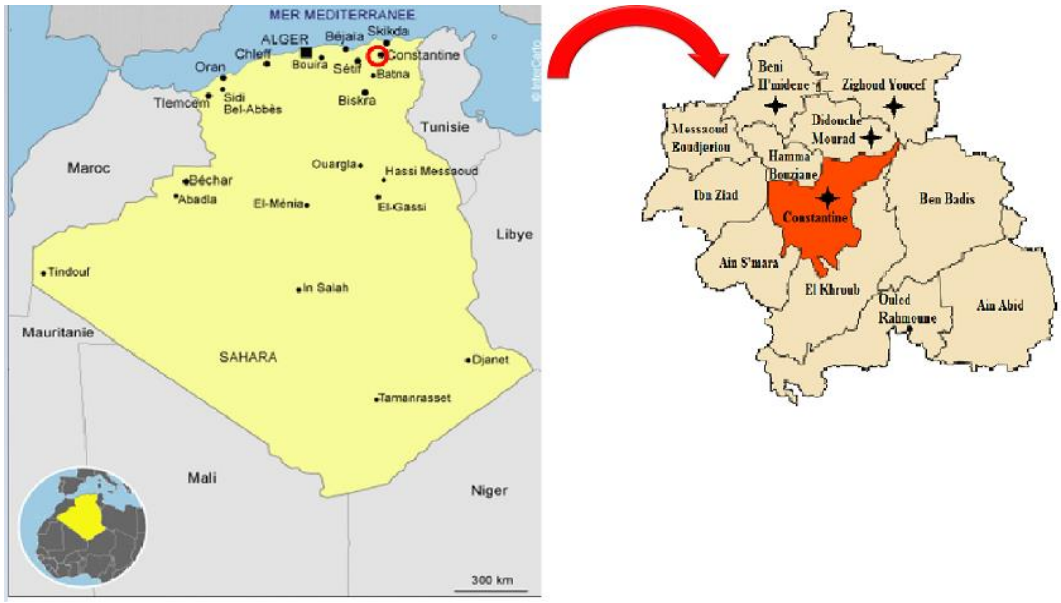

Fig 1: Location of the sampling sites.

Didouche Mourad (23° 62 'N 6° 38' E), is a municipality located to the North East of Constantine on a height of 498m and an area of 115, 70 Km^2. Six biotopes are prospected in this locality; four internal biotopesare farms (DM1, DM2, DM3, DM6) which house domestic animals, surrounded by plants and waste water points, and two externalbiotopes as isolated retaining walls (DM2, DM5) located along the roadside.

Zighoud Youcef (36° 3'N 6° 42' E) is a Municipality located 20 km north eastern the chief town of the state. It is built on an altitude of 560 meters. Five biotopesare prospected in this locality. Threebiotopesof them areexternalbiotopes in the form of retaining walls alongside the road (ZY1, ZY2, ZY5).They are surrounded by vegetation, homes, and water points. There are also two internal biotopes which are farms located on the heights of Zighoud Youcef (ZY3, ZY4) near the houses. These farms are home to cattle and sheep farms, and a strong presence of guard dogs.

Beni H'midene (36° 30'N 6° 32' E) is a municipality located to the north-west of Constantineranging over an altitude of 450 m. Five biotopesare explored in this locality, namely four internal biotopes represented by farms built of stone (BH1, BH3, BH4, BH5). They are located near homes and domestic animals and an external biotoperepresented by a retaining wall (BH2) isolated on the heights of the locality.

Chaabet Erssas (36° 17 ' 6° 37' E) is located downtown Constantine, on an altitude of 649 m. Only one outer shelter has been prospected; it is a retaining wall (CR) located near the university campus. The presence of intense vegetation and stray dogs is reported.

The traps were established identically for the three stations of Didouche Mourad, Zighoud Youcef, and Beni H'midene inside the farms and between the interstices of the retaining walls. For Chaabet Erssas station the traps were deposited in the interstices of the retaining wall only.

2.2 Acquisition of biological data

The adhesive trap technique [12], known since the work of epidemiologists [13] has been used in this prospection. This method is probably best suited to the qualitative and quantitative inventory of sand flies in the Mediterranean region [3, 14]. The adhesive trap known to be non-selective consists of a paper with particular surface (20 cm × 20 cm) soaked with castor oil. These traps are rolled in cones and placed vertically in the eventual biotopes of sand flies [15]. The biotopes are most often represented by the interstices of the retaining walls, the cracks of the stone walls and the interior walls of the stables sheltering domestic animals (sheep, cattle, chickens, and dogs).

In order to compare the densities of the sand flies between the sites surveyed in the stations, we proceeded to the regular collection of all the information about the trapping sites (altitude, orientation, temperature, and hygrometry). The used traps are recovered after two days in hot weather, and after a week in coolerweather.

A total of 26 visits, with an average of two visits per month have been done. The traps were set at a rate of 30/station, i.e. 1.2 m^2 of paper surface per station and 4.8 m^2 per visit. A total of 124.8 m^2 of paper surface was deposited. In the laboratory, samples of Phlebotomines are collected with a brush soaked in ethyl alcohol at 70° to take off the samples of the oil-soaked paper without damaging them. The collected sand flies are placed in 10% potassium hydroxide for 4 to 8 hours to lighten them.Then they are rinsed with distilled water in 6 successive baths before placing them in Marc-Andre's liquid for 1 hour. Several successive 20-minute bathsof alcohol at 70°, 90°, 95°and 100° respectively are then required to remove debris.

Using a binocular loupe, the dehydrated samples are mounted between slide and coverslip in a drop of Canada balsam diluted in xylene. For the mounting technique, the sand flies

are placed in lateral position, the head separated from the rest of the body. The head must be ventral facing in the upper position for the genus *Sergentomyia*, and in the lower position for the genus *Phlebotomus*. This position makes the observation of the cibarial and pharyngeal armatures easier. The legs are carefully spread on the ventral side and the wings on the dorsal side. The genital armatures in male and female individuals must be correctly oriented in order to highlight the elements of the diagnosis.

For identification, we focused on the determination keys of sand flies of Algeria [9, 3], whose main criteria are the forms of the pharynx, the cibariales teeth, the spermathecae as well as the male genitals armatures such as edeage, coxite, and style.

2.3 Data Processing

2.3.1 Structural and Organizational Parameters of the Identified Populations and Stands of Phlebotominae

For the exploitation of the obtained results, the characteristics of phlebotomine fauna by ecological indices have been determined. These aspects allow to know the quality of sampling, the efficiency of the trapping method and also to estimate the abundance and the dispersion of the species. The comparison of the composition of the phlebotomy populations between the biotopes in the studied stations is also discussed.

- Total wealth: corresponds to the total number of the studied population.
- Species Wealth: number of species in the stand [16].
- The relative frequency (RF) or relative abundance: percentage of individuals of a species (ni) in relation to the total number of identified individuals (N) of a population [17].

2.3.2 Statistical analysis

Correspondence Factor Analysis (CFA) is a method that allows studying the association between two qualitative variables. This method is based on inertia. The purpose of Correspondence Factor Analysis is to represent a maximum of the total inertia on the first factorial axis, a maximum of the residual inertia on the second axis, and so on until the last dimension. The number of dimensions of the representation space appears to be less than or equal to min (m1, m2) -1. Charts constitute the ultimate goal of Correspondence Factor Analysis, because they allow a much faster interpretation of the results. The principle of these methods is to leave without prior assumption on the data and to describe them by analyzing the hierarchy of the information present in the data. To do so, the factor analyzes study the inertia of the scatter plot having for coordinates the values present on the rows of the data table.

3. Results

3.1 Faunistic Inventory of Sand flies

During the present study, we chose to explore two types of Phlebotomines lodgings: internal ones represented by farms containing domestic animals and located not far from dwellings, and external ones represented by roadside retaining walls, often surrounded by vegetation and water sources. Table 1 represents a summary of the abundance of species encountered in every prospected biotope.

Table 1: Summary of the species abundance by study lodging.

Species / Biotope	Kind of biotope	*Phlebotomus perniciosus*	*Phlebotomus longicuspis*	*Phlebotomus papatasi*	*Phlebotoms perfiliewi*	*Sergentomiya minuta parroti*
DM1	internal	3	0	0	0	7
DM2	external	13	2	0	2	41
DM3	internal	120	2	0	0	180
DM4	internal	62	7	0	2	119
DM5	external	100	17	0	6	34
DM6	internal	104	4	1	0	11
ZY1	external	7	2	0	2	9
ZY2	external	13	3	0	0	3
ZY3	internal	2	2	0	0	29
ZY4	internal	14	5	0	0	8
ZY5	external	16	5	0	1	4
BH1	internal	1	0	0	1	73
BH2	external	0	0	0	0	11
BH3	internal	44	5	1	0	42
BH4	internal	56	3	0	0	56
BH5	internal	69	3	0	1	56
CR	external	28	2	0	0	126

DM : Didouche Mourad, ZY : Zighoud Youcef, BH : Beni H'miden, CR : Chaabet Erssas.

3.2 Species wealth

The results of the morphotaxonomic study on the 1540 harvested sand flies in the two types of internal and external biotopes showed the presence of two genera: *Sergentomyia* and *Phlebotomus*. In the internal biotope the genus *Sergentomyia* represents 52.16%, while the genus *Phlebotomus* represents 46.84% of the total harvests. 5 species have been identified in this type of biotope: *Sergntomyia minuta parroti* with a rate of 53.16% followed by *Phlebotomus perniciosus* with a rate of 43.46%, the dominance of these two species showed that others species were less abundant with *Phlebotomus longicuspis* (2.84%), *Phlebotomus perfiliewi* (0.37%), and *Phlebotomus papatasi* (0.16%).

In the external biotope, the genus *Sergentomyia* represents 51.01%, and the genus *Phlebotomus* represents 48.99% of the total harvests. In contrast to the first type of biotopes, only 4 species were found: *Ssergentomyia minuta parroti* with 51.01%, *Phlebotomus perniciosus* with 39.60%, the other two species are present with low values *Phlebotomus longicuspis* (6.94%), and *Phlebotomus perfiliewi* (2.46%) while the species *Phlebotomus Papatasi* is completely absent.

Figures 2 and 3, illustrate the relative frequencies of the species encountered in the two types of biotopes studied internally and externally.

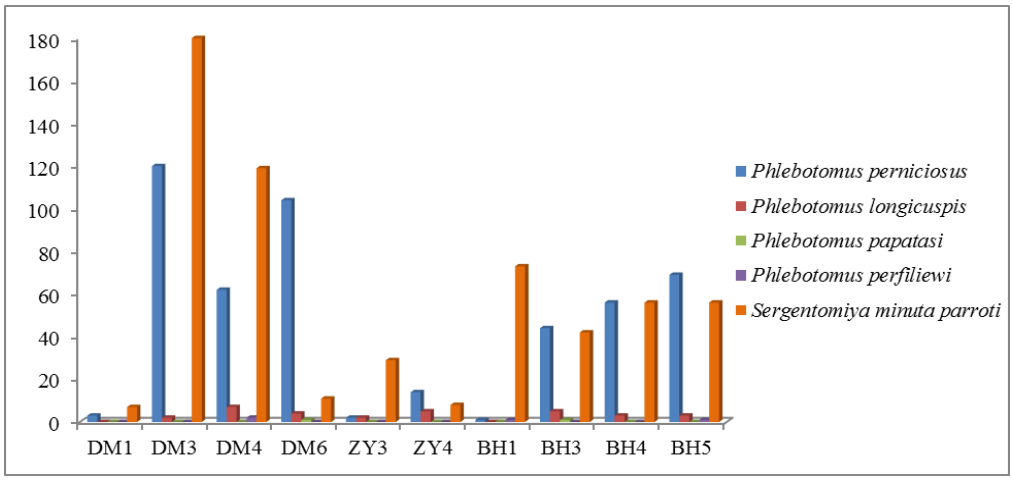

Fig 2: Species wealth of the studied internal biotopes.

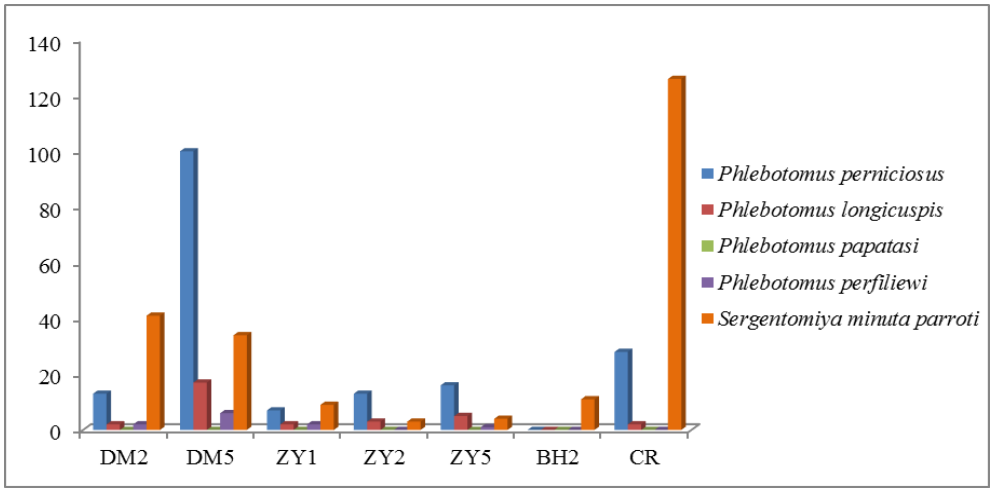

Fig 3: species wealth of the studied external biotopes.

3.3 Correspondence Factor Analysis (CFA)

The data set corresponds to a matrix of 56 samplings and 5 species that had been subject to a correspondence factor analysis (CFA), with the aim of highlighting different assemblages of sand flies as well as the special species of each assemblage. The CFA provides 76.59%, 16.61%, and 4.88% respectively of the cloud inertia for the three factorial axes.

These results indicated the predominant influence of the nature of surveyed biotopes (internal biotope, external biotope) on the Phlebotominae fauna. The interpretation of the principal axes (biotopes) can be performed indirectly according to the species with a strong relative contribution to the eigenvalues of each axis. Given their nature and their chorology the examination of the factorial maps related to the axes 1-3 (Fig. 4) leads to discriminate 5 assemblages well separated from each other.

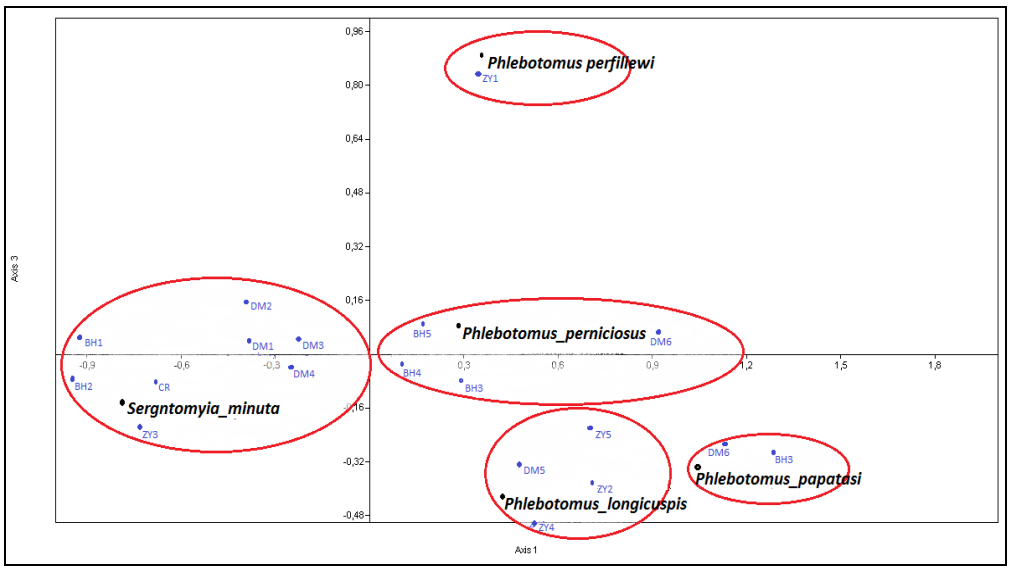

Fig 4: Presentation of the species in the F1-F3 plane of the Correspondence Factorial Analysis

3.3.1 Ascending Hierarchical Clustering (AHC)

The contribution of the assemblages was largely facilitated by the ascending hierarchical clustering calculated from the Euclidean distances between the species and the records according to the first 3 scores of the AFC (Fig. 5).

The hierarchical clustering leads to describe the total number of assemblies that was 5, and their composition which comes as follows:

1. ZY1- *Phlebotomus perfiliewi*
2. DM5, ZY5, ZY2, ZY4- *Phlebotomus longicuspis*
3. DM6, BH3, BH4, BH5- *Phlebotomus perniciosus*
4. CR, DM1, DM3, DM4, DM2, ZY3, BH1, BH2- *Sergentomyia minuta parroti*
5. DM6, BH3- *Phlebotomus papatasi*

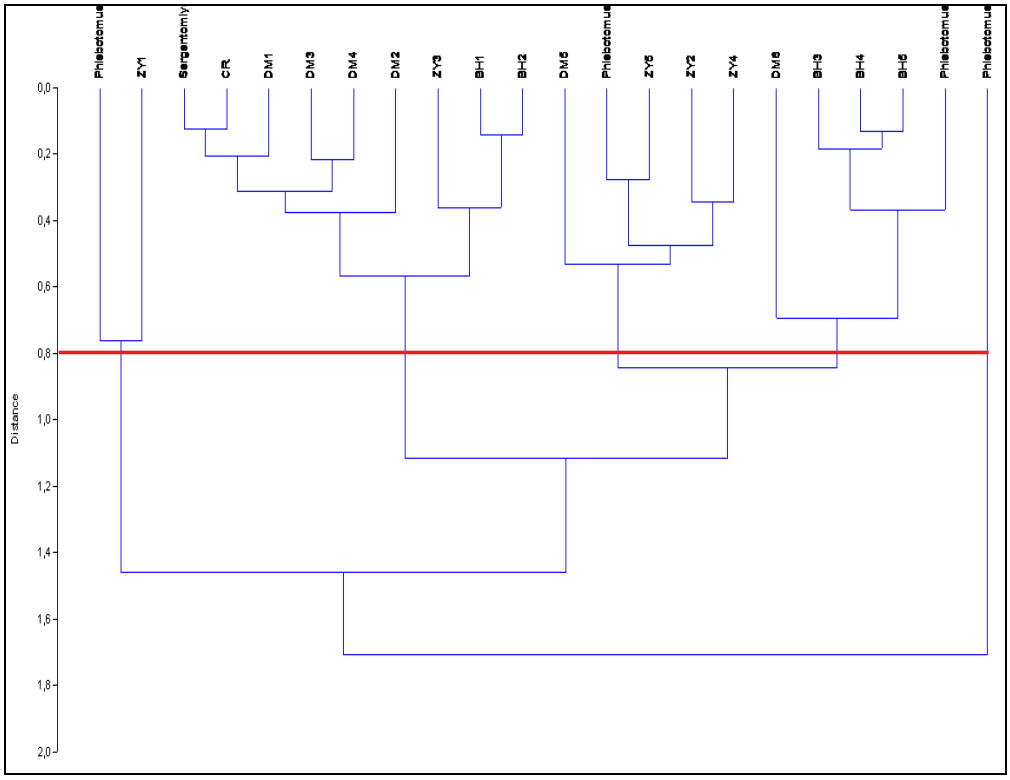

Fig 5: Representation of the ascending hierarchical clustering (AHC) of phlebotominae assemblages.

4. Discussion

During the present investigations, 1540 sandflies were captured in the period from April 2013 to October 2013 and from April 2014 to October 2014. These periods correspond to the activity of adult sandflies in temperate zones [18]. This population of sandflies is characterized by low species wealth. In fact, 5 of the 23 species of sand flies known in Algeria [10] have been surveyed. This low species wealth is already reported in Constantine by [19], in urban areas and [20]; the authors report the presence of 4 and 6 speciesrespectively. In the region of Mila, [21] reports the presence of 4 species, while in the region of Skikda, [22] reports the presence of 5 species.

It is noteworthy that the absence of certain species is explained by the fact that the traps have not been placed in their natural habitats [23] on the one hand and that species are subject to bioclimatic stages other than those in this study on the other hand [24].

This study reveals the presence of the two genera of *Phlebotominae* known in Algeria: *Sergentomyia* and *Phlebotomus*, in both types of internal and external biotopes. It also reveals the presence of 5 species in the internal biotopes: *Sergentomyia minuta parroti* (53.16%), *Phlebotomus perniciosus* (43.46%), *Phlebotomus longicuspis* (2.84%), *Phlebotomus perfiliewi* (0.37%) and *Phlebotomus papatasi* (0.18%). In the external biotopes, four species exist: *Sergentomyia minuta parroti* (51.01%), *Phlebotomus perniciosus* (39.60%), *Phlebotomus longicuspis* (6.94%), *Phlebotomus perfiliewi* (2.46%). The present study revealed the absence of the species *Phlebotomus papatasi* in this type of biotopes.

The species *Phlebotomus perniciosus* characterizes, particularly, eastern Algeria, but is found in all bioclimatic stages with a relatively high frequency in humid areas [15]. *Phlebotomus perniciosus* is considered to be the known vector of human visceral leishmaniasis due to *leishmania infantum*. In the Mediterranean basin, this species stings both humans and dogs [25]. For the genus *Phlebotomus* the species *Phlebotomus perniciosus* is the most abundant in both types of internal and external biotopes with respective rates of 43.46% and 39.60%. This same finding is reported by [26]. These authors report 77% of the total catches making it the best represented species of the studied population. This result also reveals a slight increase in the abundance rate of this species in the internalbiotopes; this is mainly due to its endophilic and anthropozoophilic nature [27]. But its presence in almost all of our prospectedbiotopes (stables, houses, and retaining walls) leads us to suppose an exophilic character of the species *Phlebotomus perniciosus*.

Phlebotomus longicuspis is reported in the Algerian Tell [25], on the Saharan stage [15] but more particularly on the highlands in the arid and per arid stages, in addition to rural and wild areas [9]. *Phlebotomus longicuspis* is the second species surveyed in the genus *Phlebotomus* with close values in the internal biotopes (2.84%) and the external ones (6.94%). In spite of its endophilic character, one can speak of a preference for the external biotopes as well.

Phlebotomus perfiliewi is a species absent in arid zones [7, 15], but well present in semi-arid bioclimatic stages [28], and especially in Constantine where it reaches its maximum development threshold in August [19]. This species is present

in both types of biotopes during our investigation, but it is 2.46% more abundant in the internal biotopes compared to the external ones 0.37%. The same findings were revealed during the investigations conducted by [19] inside an urban apartment. The obtained results reveal a very high rate of 75.7% of *Phlebotomus perfiliewi*. The same thing applies to CDC catches taken inside the stables by [22] who reported 57.69% of *Phlebotomus perfiliewi*. Similarly, the endophilic character of this speciesis confirmed to be more abundant inside than outside [22].

Phlebotomus papatasi is the known vector of zoonotic cutaneous leishmaniasis due to *leishmania major* [29, 30] and is particularly abundant throughout the north Saharan steppe [9]. This exceptional presence can be explained by the movement of sandfly populations and ecological changes [31]. In this study *Phlebotomus papatasi* species was only found in the internalbiotopes with a low rate of 0.18%. It is also noteworthy that the external biotopes do not provide a favorable environment for the development of this anthropophilic and endophilicspecies [32]. Results reported by [22] with 0.04% of catches, [33] with 0.04% of catches also and [20] with 2.2% of catches, confirm the rarity of *Phlebotomus papatasi* on the semi-arid floor and even exceptionally in the sub-humid stage [34].

Sergentomyia minuta parroti, known to be the most abundant sandfly in Algeria [32], colonizing the most varied biotopes [35]. With high densities at sub-humid and humid stages [36, 37], it isthe most frequently encountered species in this investigation, whether in the internal or external biotopes with respective rates of 43.46% and 39.60% [22], with a density of (69.82%) of the species *Sergentomyia minuta parroti* on the wet floor. According to [9], these results can be explained by the fact that *Sergentomyia minuta parroti* is widely distributed in both urban and wilderness areas. Based on these findings, an endophilic and exophilic character of this species can be assumed.

In conclusion, it has been found that the characters of andophilia and exophilia play a very important role in the distribution of sandfly specis of phlebotominae, but more biotopes should be explored to confirm this hypothesis.

5. Acknowledgement

Authors would like to Mrs. Sélima BERCHI for her invaluable help and her daily availability. Author are indebited to staff of the Laboratory of Biosystematics and Ecology of Arthropods for all the materials provided.

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