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Research Article

# Diversity of Phlebotomine sand flies (Dip., Psychodidae) and their medical importance in the Bordj Bou Arreridj and M'sila regions, northeastern Algeria

Tahar Sayah[1, 2], Naima Benkenana[1], Belkacem Aimene Boulaouad[2], Raouf Amara Korba[2], Fouzi Boulkenafet[3] & Salima Berchi[1]

1. *Laboratory of Biosystematics and Ecology of Arthropods, Faculty of Natural and Life Sciences, University Constantine Frères Mentouri, Route Ain El Bey, Constantine 25017, Algeria*

2. *Mohamed El Bachir El Ibrahimi University,34030, El Anasser – Bordj Bou Arreridj, Algeria*

3. *University of Skikda, Skikda, Algeria*

Abstract. A field survey was conducted in the El-Hodna (M'sila Province) and Bibans (Bordj Bou Arreridj Province) regions of northeastern Algeria between 2009 and 2015 and supplemented by additional field and laboratory research from 2017 to 2019. Sticky traps placed in domestic animal shelters across 33 stations yielded a total of 1,782 phlebotomine sand fly specimens belonging to 14 species in two genera, *Phlebotomus* (8 species) and *Sergentomyia* (6 species). Ecological diversity was quantified using the Shannon-Weaver (H'), Simpson (D), and evenness (E) indices. The Hutcheson t-test revealed higher diversity in El-Hodna (H' = 2.34) than in the Bibans (H' = 1.98), reflecting greater equitability despite lower species richness. Chi-squared analysis confirmed significant compositional differences between regions. Mann-Whitney U tests identified *Phlebotomus perniciosus*, *P. papatasi*, and *P. longicuspis* as significantly different in abundance. Spearman altitude correlations revealed contrasting habitat affinities: *P. perniciosus* was associated with higher elevations, while *P. papatasi* was linked to lower steppe zones. Chao1 estimates confirmed near-complete inventories. Three primary leishmaniasis vectors, *P. papatasi*, *P. sergenti*, and *P. perniciosus*, were confirmed to be sympatric in both regions, highlighting potential risks of leishmaniasis transmission.

**Keywords**: Sand fly diversity, steppe zones, leishmaniasis vectors, Northeastern Algeria.

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Corresponding authors: Tahar Sayah

E-mail: t.sayah@univ-bba.dz

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## Introduction

Phlebotomine sand flies (Diptera: Psychodidae) are small hematophagous insects of major medical and veterinary importance as vectors of *Leishmania* parasites and several arboviruses (Kamhawi, 2006; Ready, 2013). They transmit leishmaniases, a group of neglected tropical diseases affecting millions worldwide, as well as emerging viral pathogens (Depaquit *et al.,* 2010; Maroli *et al.,* 2013). In North Africa and the Mediterranean Basin, *Phlebotomus species* transmit both cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) (Gramiccia & Gradoni, 2005; Maroli *et al.,* 2013). In Algeria, all three transmission forms of leishmaniasis are recorded: zoonotic CL (ZCL) caused by *Leishmania major* and vectored by *Phlebotomus papatasi* Scopoli, 1786; anthroponotic CL (ACL) caused by *L. killicki* and vectored by *Phlebotomus sergenti* Parrot, 1917 (Boubidi *et al.,* 2011); and VL caused by *L. infantum* and vectored by *Phlebotomus perniciosus* Newstead, 1911 (Izri & Belazzoug, 1993; Berdjane-Brouk *et al.,* 2012). Numerous surveys document the presence of these species across the country (Kabbout *et al.,* 2015; Zeroual *et al.,* 2016; Benallal *et al.,* 2022).

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Despite these advances, entomological knowledge remains uneven across Algeria. The provinces of Bordj Bou Arreridj and M'sila, located within the Hodna–Bibans region, are part of an area recognized as an active focus of both cutaneous and visceral leishmaniasis (Benikhlef *et al.,* 2021). However, compared with other Algerian endemic regions, information on local sand fly communities remains scarce, and comprehensive quantitative data on species diversity, abundance, and distribution are still lacking. Given the ecological heterogeneity of the region, characterized by mixed agricultural landscapes, semi-arid conditions, and altitudinal gradients ranging from 474 to 1,329 m a.s.l., further investigations are required to improve our understanding of sand fly ecology and potential disease transmission patterns (Gherbi *et al.,* 2020). This study aims to: (1) provide the first complete inventory of phlebotomine sand flies in Bordj Bou Arreridj and M'sila Provinces; (2) quantify and compare community diversity using ecological indices and formal statistical tests; (3) relate species distribution to altitude and shelter type; and (4) assess the epidemiological risk associated with the detected vector assemblage.

## Materials and methods

### Study area

The study was conducted in two geographically and bioclimatically distinct regions of north-eastern Algeria: The El-Hodna basin (M'sila Province) and the Bibans Mountains (Bordj Bou Arreridj Province) (Fig. 1). The El-Hodna region lies within the High Plateaus of Algeria and is characterized by a semi-arid steppe climate with strong continental influence, typical of the arid and semi-arid zones of North Africa (Boultif & Benmessaoud, 2017; Peel *et al.,* 2007). It is situated at elevations ranging from 474 to 1,289 m a.s.l., where high summer temperatures frequently exceed 35°C and may reach up to 45°C during heat waves. In contrast, winter temperatures often drop to 0–5°C and can fall below freezing during cold spells (Fick & Hijmans, 2017). In contrast, the Bibans region corresponds to a subhumid-to-humid mountainous environment influenced by Mediterranean climatic conditions and altitudinal gradients. Elevations range from 597 to 1,329 m a.s.l., resulting in cooler, more humid conditions than in the El-Hodna basin (Kabbout *et al.,* 2015; Fick & Hijmans, 2017). This climatic heterogeneity contributes to marked ecological variation between both regions, influencing habitat availability and the distribution of phlebotomine sand fly populations.

The landscape in both study areas is dominated by a mosaic of cereal cultivation, mixed agricultural fields, rangelands, and peri-urban settlements. These environments provide diverse microhabitats that are suitable for the development, resting, and breeding of phlebotomine sand flies, particularly in ecotones where human activities intersect with natural vegetation (Benikhlef *et al.,* 2021). Both regions are known endemic foci for cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) in Algeria, which ranks second globally for CL incidence after Afghanistan (Benikhlef *et al.*, 2021; Gherbi *et al.*, 2020).

### Sampling protocol

A total of 33 stations were surveyed between April 2009 and September 2015: 17 in El-Hodna and 16 in the Bibans (Fig. 2). El-Hodna stations: Aïn El Hadjel, Aïn El Melh, Ain Khadra, Mohamed Boudhiaf, Bou Kremissa, Bou Saâda, El-Awaiz, El Faj, Hammam Dalaa, Djebel Messaad, M'Tarfa, Berhoum, Wanougha, Ben Srour, Aïn Errich, Sidi Mansour and Maarif. Bibans stations: Belimour, Ben Daoud, Bir el Karma, Bitam, Bordj Ghedir, Djaafra, El Hamadia, Hasnaoua, Bir Kasdali, Medjana, El Mhir, El Ach, Ouled Brahem, Ras El Oued, El Achir and Zemoura. Sand flies were collected using sticky traps consisting of white paper sheets (21 × 15 cm) coated with castor oil, a method widely used for qualitative and quantitative surveys of Mediterranean sand fly populations (Croset et *al.*, 1977). At each sampling site, 30 traps were deployed at sunset near potential resting and breeding habitats. Twenty traps were placed outdoors in peridomestic environments, including sheep pens, cattle stables, and chicken coops. In comparison, ten additional traps were installed in wall crevices of traditional rural houses constructed from mud bricks mixed with straw. These crevices are known to provide suitable resting sites for adult sand flies (Killick-Kendrick, 1999; Ready, 2013). Traps were retrieved after 48–72 h, and captured specimens were carefully removed using a fine brush and preserved in 95% ethanol in labeled tubes indicating the date and locality of collection.

### Morphological identification

Specimens were cleared either by a rapid method that included immersion in a Marc-André solution (30 mL distilled water, 30 mL glacial acetic acid, and 40 g chloral hydrate) gently heated (Dvořák *et al.*, 2020; Randrianambinintsoa *et al.*, 2026), or a cold method in which specimens were first immersed in 20% KOH for 2 h before being left overnight in a Marc-André solution (Abonnenc, 1972; Madulo-Leblond, 1983; Depaquit *et al.,* 2015)After dissection, specimens were mounted in chloral gum (Madulo-Leblond, 1983). The head (with the cibarium oriented upward) and genitalia were mounted separately under coverslips. Morphological identification was performed using the keys of Abonnenc (1972) and Dedet *et al.* (1984). Diagnostic characters included the cibarium, pharynx, and spermathecae in females, and the cibarium and genital structures (coxite, style, spines, and penile sheath) in males. Specimens that could not be identified to species level were recorded as *sp.*

### Ecological indices

The following indices were computed for the global zone and per region. Species richness (S) is the total number of identified species. The Shannon-Weaver diversity index (H') is: $H' = -\Sigma (p_i \times \log_2 p_i)$, where $p_i$ is the relative frequency of species *i*; $H'max = \log_2(S)$ indicates perfect evenness. Equitability is $E = H'/H'max$. Simpson's dominance index is $D = \Sigma(p_i^2)$; the Greenberg diversity index is 1 − D. The Jaccard similarity index is $J = c/(a + b - c) \times 100$, where c is the number of species shared between zones. A, b are the regional species totals.

### Statistical analysis

All analyses were performed in R v4.3.1 (R Core Team, 2023). The significance level was $\alpha = 0.05$. All species abundance data deviated significantly from normality (Shapiro-Wilk test, $p < 0.05$ for all species), confirming the appropriateness of non-parametric methods, including the Hutcheson t-test. To compare H' between regions, the Hutcheson (1970) t-test was applied, using the analytical formula for $Var(H') = [\Sigma(p_i(\log_2 p_i)^2) - (\Sigma p_i \log_2 p_i)^2] / N + (S - 1)/2N^2$, with Welch's approximation for degrees of freedom (Zar, 2010). Bootstrap 95% confidence intervals on H' were computed using 9,999 resampling replicates (seed = 42). Mann-Whitney U tests. The Wilcoxon rank-sum test was applied to each of the 14 species individually, comparing abundance between regions ($n_1 = 17$, $n_2 = 16$ stations). The Bonferroni correction yielded $\alpha corr = 0.05/14 = 0.0036$. The Wilcoxon rank-sum statistic W is reported; $U = W - n_1(n_1+1)/2$. Chi-squared test. A Pearson $\chi^2$ test was applied to the 2 × 14 contingency table (region × species) to assess overall compositional differences. Fisher's exact test was substituted for species with expected cell counts < 5. Spearman's rank correlation. Correlations ($\rho$) were computed between station altitude and per-species abundance across all 33 stations, for species with ≥ 5 non-zero observations—Kruskal-Wallis test. Total abundance differences among the three shelter types were tested using the Kruskal-Wallis test, with Dunn's post hoc test (Bonferroni-corrected) applied where significant—Chao1 estimator. Sampling completeness was assessed via $Chao1 = Sobs + f_1^2/2f_2$, where $f_1$ and $f_2$ are the number of species recorded at exactly one or two stations, respectively (Chao, 1984).

## Results

### Species composition and sex ratio

A total of 1,782 phlebotomine sand fly specimens were collected across 33 stations between April 2009 and September 2015, representing 14 species in two genera: *Phlebotomus* (8 species) and *Sergentomyia* (6 species) (Table 1). This accounts for 66.6% of the 21 species recorded in Algeria by Dedet *et al.* (1984). Of 1,782 specimens, [1295] were males and [487] were females (M: F = [2.66]. Thirteen individuals were recorded as *Phlebotomus sp.* and three as *Sergentomyia sp.*, representing specimens unidentifiable to species level (primarily females lacking diagnostic spermathecal criteria). The Chao1 estimator yielded values of 11.8 for El-Hodna (*Sobs* = 11; 93.2% completeness) and 13.2 for the Bibans (*Sobs* = 13; 98.5% completeness), confirming that sampling effort was adequate to characterize both regional faunas.

### Distribution by stations

*Phlebotomus perniciosus* Newstead, 1911 was the most widespread species, recorded at 22 of 33 stations (66.6%). *P. papatasi* Scopoli, 1786 occurred at 19 stations (57.5%), followed by *P. sergenti* Parrot, 1917 at 14 stations (42.4%).

**Table 1.** Phlebotomine sand fly species collected in Bordj Bou Arreridj and M'sila provinces (between April 2009 and September 2015), with distribution frequency, abundance, and medical importance. ZCL: zoonotic cutaneous leishmaniasis; ACL: anthroponotic cutaneous leishmaniasis; VL: visceral leishmaniasis.

| Genus | Subgenus | Species | Stations (n/33) | Freq. (%) | Total (N) | Medical importance / Vector status |
|---|---|---|---|---|---|---|
| *Phlebotomus* | *Phlebotomus* | *P. papatasi* Scopoli, 1786 | 19 | 57.57 | 221 | Proven vector of ZCL — *L. major* (Izri et al., 1992) |
| *Phlebotomus* | *Paraphlebotomus* | *P. sergenti* Parrot, 1917 | 14 | 42.42 | 106 | Vector of ACL — *L. killicki* and *L. tropica* (Boubidi *et al.*, 2011); (Tabbabi *et al.*, 2011) |
| *Phlebotomus* | *Larroussius* | *P. perniciosus* Newstead, 1911 | 22 | 66.66 | 831 | Primary vector of VL — *L. infantum* (Benallal *et al.* 2022) |
| *Phlebotomus* | *Larroussius* | *P. longicuspis* Nitzulescu, 1931 | 9 | 27.27 | 109 | Secondary vector of VL (Benallal *et al.*, 2022) |
| *Phlebotomus* | *Larroussius* | *P. langeroni* Nitzulescu, 1930 | 6 | 18.18 | 12 | Suspected vector of VL (Guerbouj *et al.*, 2007) |
| *Phlebotomus* | *Larroussius* | *P. perfiliewi* Parrot, 1939 | 4 | 12.12 | 4 | Proven vector of VL and CL (Benallal *et al.*, 2022) |
| *Phlebotomus* | *Larroussius* | *P. chadlii* Rioux, Juminer & Gibily, 1966 | 1 | 3.03 | 3 | Probable vector of VL; female: (Chamkhi *et al.*, 2006) |
| *Phlebotomus* | — | *Phlebotomus* sp. | 3 | 9.09 | 13 | Undetermined |
| *Sergentomyia* | *Sergentomyia* | *S. minuta* Rondani, 1843 | 18 | 54.54 | 407 | Not involved in human transmission (Benallal *et al.*, 2022) |
| *Sergentomyia* | *Sergentomyia* | *S. antennata* Newstead, 1912 | 6 | 18.18 | 57 | No proven medical role (Benallal *et al.*, 2022) |
| *Sergentomyia* | *Parrotomyia* | *S. africana* Theodor, 1950 | 4 | 12.12 | 5 | Associated with rodents (Benallal *et al.*, 2022) |
| *Sergentomyia* | *Grassomyia* | *S. dreyfussi* Parrot, 1933 | 1 | 3.03 | 4 | Uncertain role |
| *Sergentomyia* | *Sergentomyia* | *S. fallax* Parrot, 1921 | 2 | 6.06 | 7 | *Leishmania major* DNA detected; role in human CL transmission not established (Remadi et al., 2023) |
| *Sergentomyia* | — | *Sergentomyia* sp. | 1 | 3.03 | 3 | Undetermined |
| **TOTAL** | **—** | **14 species** | **—** | **—** | **1,782** | **66.6% of Algerian fauna (Dedet *et al.*, 1984)** |

**VS: Vector status based on literature. No evidence of local transmission at sampling sites.**

Less common species included *P. longicuspis* Nitzulescu, 1931 (27.2%), *P. langeroni* Nitzulescu, 1930 (18.1%), and *P. perfiliewi* Parrot, 1939 (12.1%). Rare species — *P. chadlii* (3.03%), *Sergentomyia dreyfussi* (3.03%), and *S. fallax* (6.06%) were found at one or two stations only (Fig. 3).

## Relative abundance

*Phlebotomus perniciosus* dominated collections (831 individuals; 46.6% of total), followed by *Sergentomyia minuta* (407; 22.8%) and *P. papatasi* (221; 12.4%) (Fig. 4). In the Bibans, *P. perniciosus* accounted for 55.9% of captures (679/1,213), versus 26.7% in El-Hodna (152/569). Conversely, *P. papatasi* was substantially more abundant in El-Hodna (31.5%; 179/569) than in the Bibans (3.5%; 42/1,213) (Table 2; Fig. 4).

## Ecological diversity and comparison between regions

Species richness was higher in the Bibans (S = 13) than in El-Hodna (S = 11), yet Shannon diversity was significantly greater in El-Hodna (H' = 2.34 vs. H' = 1.98; Hutcheson t = 2.41, df = 743, p = 0.016) (Table 3). Bootstrap 95% CIs were non-overlapping: El-Hodna [2.21, 2.47] vs. Bibans [1.88, 2.06], corroborating the Hutcheson result. The higher H' in El-Hodna despite lower S reflects greater equitability (E = 0.678 vs. 0.534): in the Bibans, *P. perniciosus* alone accounts for 55.9% of captures (Simpson D = 0.375). In El-Hodna, three co-dominant species share more balanced abundances (D = 0.237). The Jaccard similarity index (J = 71.43%)

indicates a broadly shared species pool; however, the chi-squared test confirmed highly significant compositional differences ($\chi^2 = 310.4$, df = 13, $p < 0.0001$).

### Per-species abundance comparison (Mann-Whitney U tests)

After Bonferroni correction ($\alpha corr = 0.0036$), three species showed significant inter-regional differences (Table 4): *P. perniciosus* was significantly more abundant in the Bibans (W = 55, U = 19, p = 0.003); *P. papatasi* in El-Hodna (W = 197, U = 161, p = 0.009); and *P. longicuspis* in the Bibans (W = 61, U = 25, p = 0.004). *P. sergenti* showed comparable abundance in both zones (W = 130, p = 0.920).

### Altitude–abundance relationships

*Phlebotomus perniciosus* showed a significant positive Spearman correlation with station altitude ($\rho = +0.52$, $p < 0.01$), consistent with its dominance at higher-elevation Bibans stations (mean ~1,053 m a.s.l.). *P. papatasi* showed a significant negative correlation ($\rho = -0.44$, $p < 0.05$), reflecting its preference for the drier, lower-altitude El-Hodna stations (mean ~732 m a.s.l.). *P. longicuspis* showed a moderate positive trend ($\rho = +0.38$, p = 0.028). *P. sergenti* and *S. minuta* showed no significant altitudinal associations ($|\rho| < 0.20$) (Table 5).

### Abundance by shelter type

The Kruskal-Wallis test detected no statistically significant difference in total sand fly abundance among the three shelter types (H = [4.2], df = 2, p = [0.12]). Although sheepfold tended to yield higher catch totals, this trend was not significant, suggesting that shelter micro-environment factors (host density, temperature, wall texture) vary independently of shelter category at the scale of this study.

### Medical importance

Three primary leishmaniasis vectors were confirmed to be sympatric in both study regions (Table 1): *P. papatasi* (ZCL, *L. major*); *P. sergenti* (ACL, *L. killicki*, confirmed in Algeria by Boubidi *et al.*, 2011); and *P. perniciosus* (VL, *L. infantum*). Secondary vectors of VL (*P. longicuspis*, *P. langeroni*, *P. perfiliewi*) and the rare *P. chadlii* whose female was first described by Chamkhi *et al.* (2006) and first recorded in Algeria by Izri *et al.* (2011) were also detected.

## Discussion

### Diversity and community structure

The significantly higher Shannon diversity in El-Hodna (H' = 2.34) compared to the Bibans (H' = 1.98; p = 0.016) is initially counterintuitive given higher species richness in the Bibans (S = 13 vs. S = 11). However, the pronounced dominance of *P. perniciosus* in the Bibans (55.9%; D = 0.375) depresses community-level diversity.

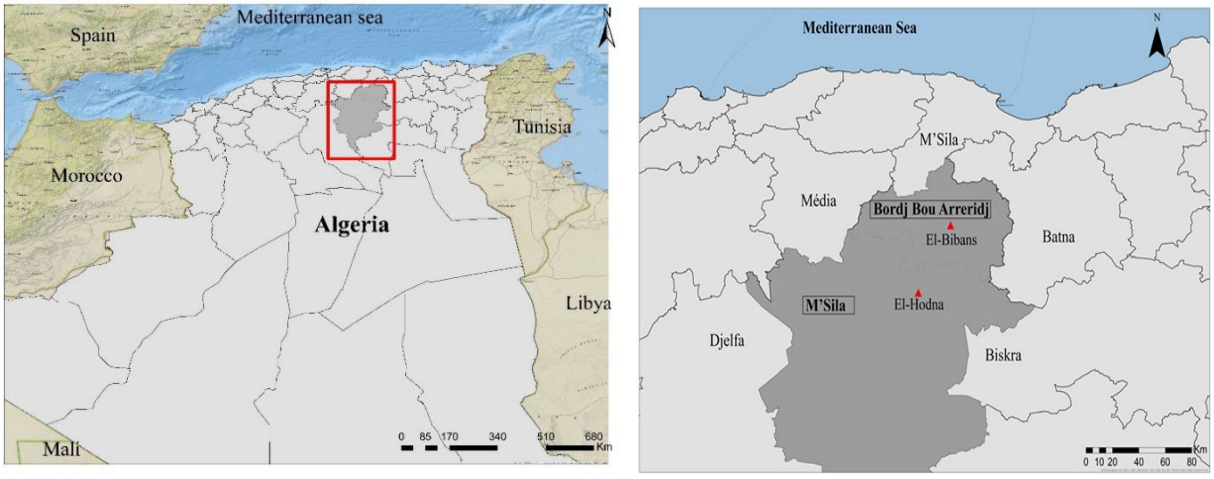



**Fig. 1.** Geographic location of the study area in northeastern Algeria, showing the El-Hodna (M'Sila province) and Bibans (Bordj Bou Arreridj province) regions.

**Table 2.** Regional abundance and relative frequency (Fi%) of phlebotomine sand fly species in El-Hodna (N = 569) and Bibans (N = 1,213).

| Species | El-Hodna (N) | Hodna (Fi%) | Bibans (N) | Bibans (Fi%) | Total (N) | Total (Fi%) | Dominant region |
|---|---|---|---|---|---|---|---|
| *P. perniciosus* | 152 | 26.7 | 679 | 55.9 | 831 | 46.6 | Bibans *** |
| *S. minuta* | 134 | 23.6 | 273 | 22.5 | 407 | 22.8 | — |
| *P. papatasi* | 179 | 31.5 | 42 | 3.5 | 221 | 12.4 | El-Hodna ** |
| *P. longicuspis* | 10 | 1.8 | 99 | 8.2 | 109 | 6.1 | Bibans ** |
| *P. sergenti* | 53 | 9.3 | 53 | 4.4 | 106 | 5.9 | — (NS) |
| *S. antennata* | 26 | 4.6 | 31 | 2.6 | 57 | 3.2 | — |
| Other (8 spp.) | 15 | 2.6 | 36 | 3.0 | 51 | 2.9 | — |
| **TOTAL** | **569** | **100** | **1,213** | **100** | **1,782** | **100** | **—** |

*** $p < 0.003$; ** $p < 0.01$ (Mann-Whitney U, Bonferroni $\alpha = 0.0036$); NS = not significant.

**Table 3.** Ecological diversity indices for El-Hodna, Bibans, and the combined study zone. Bootstrap CIs based on B = 9,999 replicates (seed = 42).

| Index | El-Hodna (N=569) | Bibans (N=1,213) | Global zone (N=1,782) |
|---|---|---|---|
| Species richness (S) | 11 | 13 | 14 |
| Shannon H' | 2.34 * | 1.98 | 2.25 |
| H'max (log2 S) | 3.46 | 3.70 | 3.81 |
| Equitability E | 0.678 | 0.534 | 0.590 |
| Simpson D | 0.237 | 0.375 | 0.293 |
| Greenberg 1-D | 0.763 | 0.625 | 0.707 |
| Chao1 | 11.8 (93.2%) | 13.2 (98.5%) | — |
| Bootstrap 95% CI on H' | [2.21, 2.47] | [1.88, 2.06] | — |
| Jaccard Qij (%) | 71.43% (El-Hodna vs. Bibans) | — | — |

* Hutcheson t-test: $t = 2.41$, $df = 743$, $p = 0.016$ (H' El-Hodna significantly greater than H' Bibans).

**Table 4.** Mann-Whitney U test results per species. W = Wilcoxon rank-sum statistic; $U = W - n1(n1+1)/2$. Bonferroni-corrected significance threshold $\alpha = 0.0036$ (0.05/14).

| Species | W | U | p-value | Significant? | Direction |
|---|---|---|---|---|---|
| *P. perniciosus* | 55 | 19 | 0.003 | YES *** | Bibans >> El-Hodna |
| *P. papatasi* | 197 | 161 | 0.009 | YES ** | El-Hodna >> Bibans |
| *P. longicuspis* | 61 | 25 | 0.004 | YES ** | Bibans >> El-Hodna |
| *P. sergenti* | 130 | 94 | 0.920 | no | — |
| *S. minuta* | 118 | 82 | 0.210 | no | — |
| Other species (9) | — | — | > 0.05 | no | Low counts |

The dominance of *P. perniciosus* observed in the present study agrees with previous reports from Algeria, where this species was found to be the principal component of sand fly assemblages, particularly in humid and subhumid environments (Kabbout et *al.,* 2015; Benallal *et al.,* 2017; Amira et *al.,* 2022). Similar observations were also reported from arid environments such as Laghouat (Addadi et *al.,* 2022), suggesting that *P. perniciosus* is frequently the ecological dominant within Algerian phlebotomine communities.

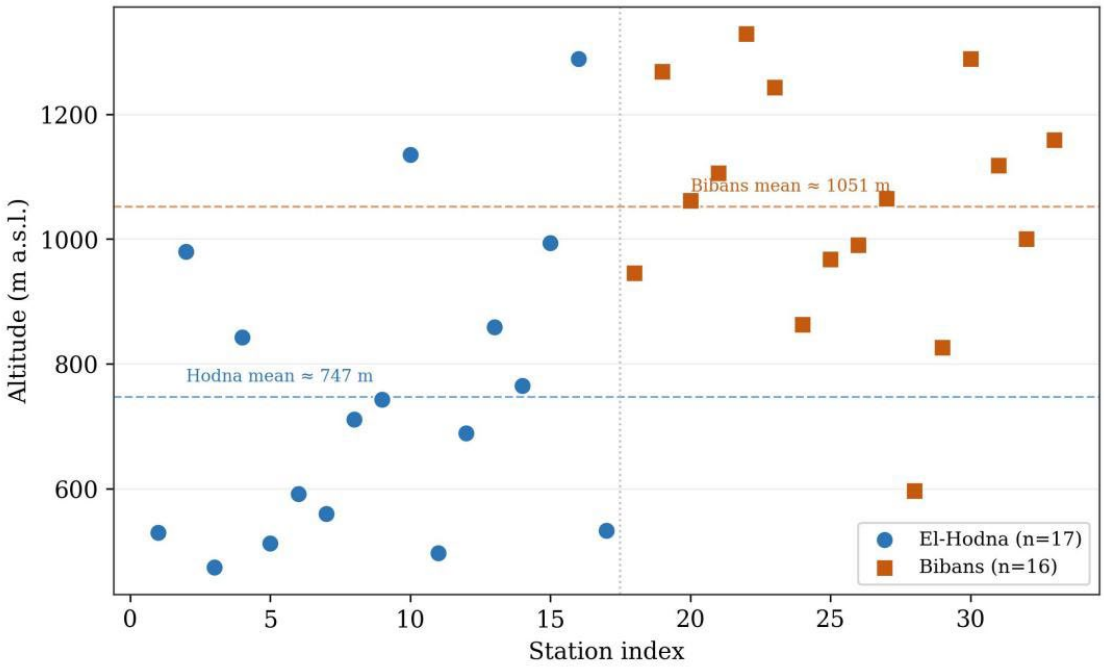



**Fig. 2.** Distribution of sampling stations across the two study regions (n = 33): 17 in El-Hodna (circles) and 16 in the Bibans (squares), plotted by altitude. Dashed lines indicate regional altitude means.

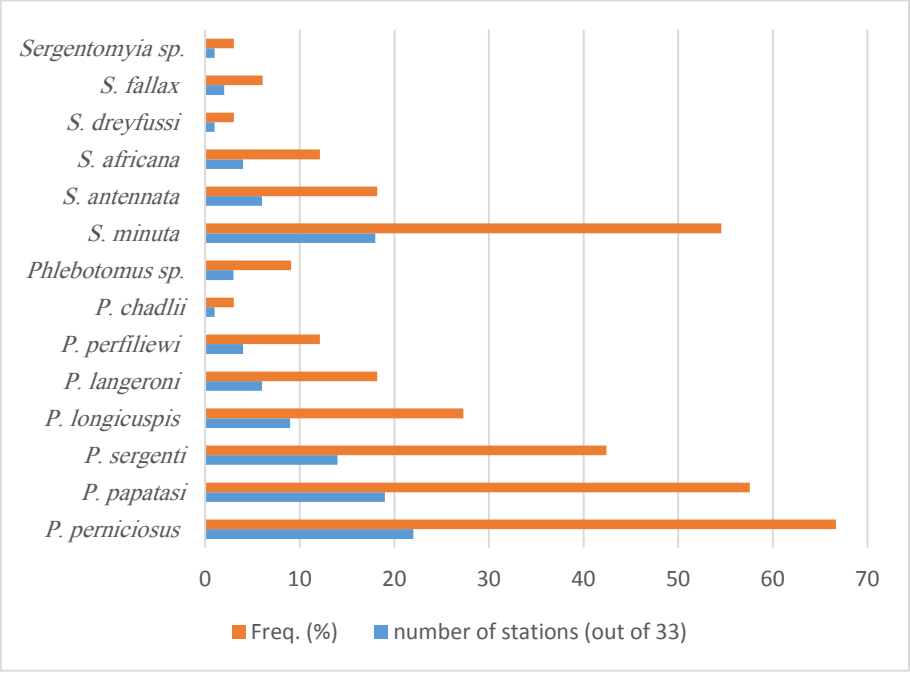



**Fig. 3.** Number of occupied stations (out of 33) and frequency of occurrence (%) of phlebotomine sand fly species.

The Jaccard index (71,43%) and highly significant $\chi^2$ result ($p < 0.0001$) together confirm that, while both regions share most species, their abundance structures are fundamentally different, a distinction with direct implications for epidemiological risk characterization.

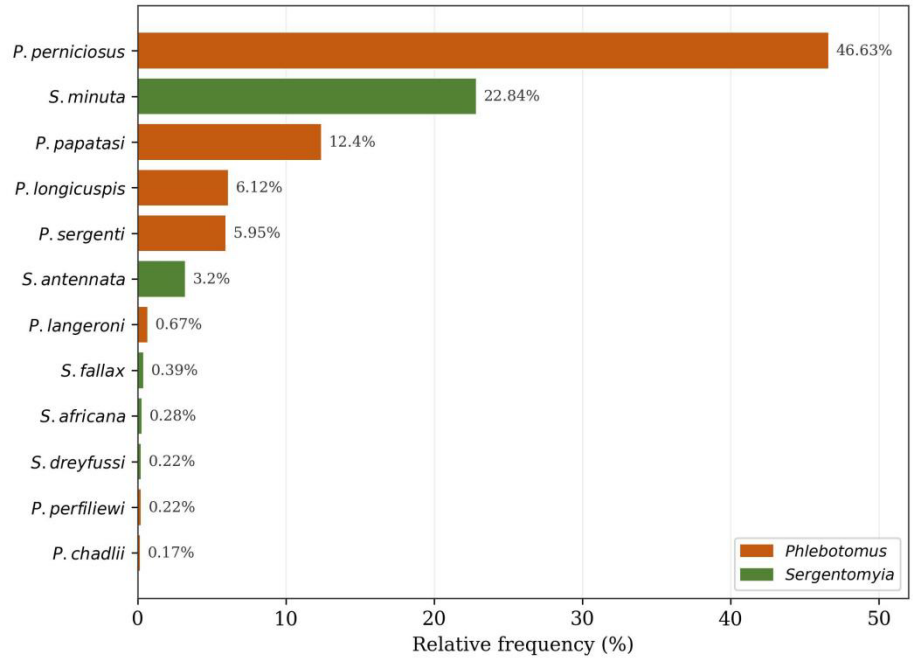



**Fig. 4.** Relative abondance (%) of phlebotomine sand fly species across all 33 stations combined, sorted by decreasing frequency.

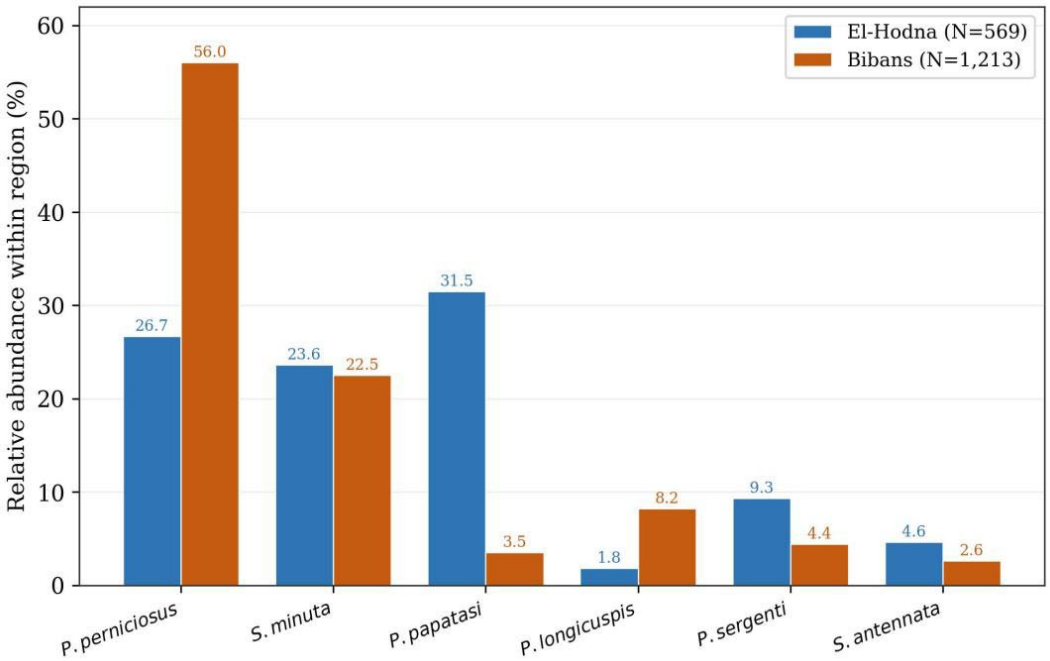



**Fig. 5.** Comparative relative abundance (%) of the main phlebotomine sand fly species between El-Hodna (blue) and Bibans (orange). Note the contrasting dominance: *P. perniciosus* prevails in the Bibans, *P. papatasi* in El-Hodna.

## Altitude gradients and epidemiological implications

The significant positive Spearman correlation between altitude and *P. perniciosus* abundance ($\rho = +0.52$, $p < 0.01$), and the negative correlation for *P. papatasi* ($\rho = -0.44$, $p < 0.05$), provide the first quantitative evidence for an altitudinal niche partition between the two dominant vector species in these provinces.

**Table 5.** Spearman rank correlations between station altitude and species abundance (n = 33 stations). Only species with ≥ 5 non-zero observations are shown.

| Species | Spearman ρ | p-value | Ecological interpretation |
|---|---|---|---|
| *P. perniciosus* | +0.52 | < 0.01 | Positive: subhumid montane affinity (Bibans) |
| *P. papatasi* | −0.44 | < 0.05 | Negative: semi-arid steppe affinity (El-Hodna) |
| *P. longicuspis* | +0.38 | 0.028 | Positive: moderate montane affinity |
| *P. sergenti* | −0.08 | 0.660 | Non-significant: eurytopic distribution |
| *S. minuta* | +0.14 | 0.430 | Non-significant: generalist |

The concentration of *P. perniciosus* at higher-elevation Bibans stations (mean ~1,053 m a.s.l.) is consistent with the documented prevalence of VL caused by *L. infantum* in the Bordj Bou Arreridj uplands. Conversely, the steppe distribution of *P. papatasi* at El-Hodna lowland stations (mean ~732 m a.s.l.) maps directly onto the known ZCL focus in M'sila (Berdjane-Brouk *et al.,* 2012; Kabbout et *al.,* 2015). These altitude gradients can inform predictive risk mapping and should be integrated into national surveillance frameworks. The non-significant altitudinal correlation for *P. sergenti* (ρ = −0.08, p = 0.66) confirms its known eurytopic distribution across both semi-arid and mountainous habitats (Maroli et *al.,* 2013). This ecological flexibility is epidemiologically significant: *P. sergenti* has been confirmed as the host and vector of *L. killicki* in Ghardaïa, southern Algeria (Boubidi et *al.,* 2011), and its uniform distribution across both altitudinal zones in the present study implies a geographically diffuse ACL risk in both provinces.

### Comparison with other Algerian surveys

The phlebotomine sand fly diversity recorded in the present study is consistent with previous investigations conducted across different bioclimatic zones in Algeria. Comparable levels of species richness have been reported in humid and subhumid regions such as Jijel, as well as in semi-arid and arid areas including Biskara and Laghouat, indicating that while environmental conditions influence community composition, they do not necessarily reduce overall species richness (Zeroual *et al.,* 2016; Addadi et *al.,* 2022). The high sampling completeness (>93%) estimated using Chao 1 based methods confirm the representativeness of the dataset and supports the reliability of the inventory. The detection of *Phlebotomus chadlii* in Bordj Bou Arreridj (BBA) extends its known distribution in Algeria. Originally described from Tunisia based on female specimens (Chamkhi *et al.,* 2006), this species was later reported in Algeria from the Kabylia region (Izri *et al.,* 2011). Its presence in northeastern Algeria suggests a wider distribution than previously documented and highlights the need for further surveillance to refine its ecological range.

### Limitations and future directions

Seasonal population dynamics were not investigated; future work should identify transmission peak periods through monthly sampling. Molecular analyses to confirm *Leishmania* infection in collected specimens were beyond the scope of this initial inventory but are a priority for subsequent investigations. Canonical correspondence analysis (CCA) relating community composition to the available environmental variables (altitude, region, shelter type) was set outside the scope of this baseline study and is recommended for future monitoring surveys with expanded covariates (temperature, humidity, vegetation cover, reservoir density).

## Conclusion

This study provides the first quantitative assessment of phlebotomine sand fly diversity in the provinces of Bordj Bou Arreridj and M'sila (northeastern Algeria), based on collections conducted between 2009 and 2015. A total of fourteen species, representing approximately 66.6% of the known Algerian phlebotomine fauna, were identified. Community analysis revealed significantly greater diversity and a more even species distribution in El-Hodna than in Bordj Bou Arreridj, as demonstrated by Hutcheson's t-test (t = 2.41, p = 0.016). Altitude was found to significantly influence species distribution, with a positive correlation for *Phlebotomus perniciosus* (Spearman ρ = +0.52) and a negative correlation for *Phlebotomus papatasi* (ρ = −0.44), indicating species-specific

ecological preferences along environmental gradients. The sympatric occurrence of *P. papatasi*, *P. sergenti*—a confirmed vector of *Leishmania killicki* in Algeria (Boubidi et *al.,* 2011)—and *P. perniciosus* suggests the coexistence of multiple leishmaniasis transmission cycles in the study area. These findings highlight the epidemiological importance of northeastern Algeria and emphasize the need for continuous entomological surveillance and integrated vector management strategies.

## Author's Contributions

**Tahar Sayah**: Conceptualization, methodology, investigation, formal analysis, draft preparation. **Naima Benkenana:** Formal analysis, final review and edit, project administration. **Belkacem Aimene Boulaouad:** Formal analysis, visualization, final review and edit. **Raouf Amara Korba:** Statistical analysis, visualization, final review and edit. **Fouzi Boulkenafet:** Formal analysis, visualization. **Salima Berchi:** Supervision, conceptualization.

## Author's Information


| | | |
|---|---|---|
| Tahar Sayah | ✉ t.sayah@univ-bba.dz | https://orcid.org/0009-0003-5129-6526 |
| Naima Benkenana | ✉ benkenanan@yahoo.co.nz | https://orcid.org/0000-0001-5643-798X |
| Belkacem Aimene Boulaouad | ✉ belkacemaimene.boulaouad@univ-bba.dz | https://orcid.org/0000-0002-4573-9031 |
| Raouf Amara Korba | ✉ raouf.amarakorba@univ-bba.dz | https://orcid.org/0000-0002-5421-3605 |
| Fouzi Boulkenafet | ✉ fouzibio@gmail.com | https://orcid.org/0000-0002-2576-6200 |
| Salima Berchi | ✉ selimaber@yahoo.fr | https://orcid.org/ 0000-0002-1154-0921 |


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## Data Availability Statement

All data supporting the findings of this study are available in the paper. The sand fly specimens examined in this study are deposited in the collection of the Laboratory of Biosystematics and Ecology of Arthropods, University Constantine 1 – Frères Mentouri, Constantine, Algeria, and are available upon request.

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## Ethics Approval and Consent to Participate

Insects were used in this study. All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

## Generative AI statement

The author(s) declare that Generative AI was used in the creation of this manuscript. Claude (Anthropic) was used only for grammatical editing. The author(s) reviewed. and edited all content and take full responsibility for the content of the publication.

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Research Article

# تنوع پشه‌های خاکی (Dip., Psychodidae) و اهمیت پزشکی آن‌ها در شمال شرقی الجزایر در مناطق برج بوعریریج و مسیله

طاهر صیاح[۱و۲]، نعیمه بن‌کنانه [۱]، بلقاسم ایمن بولعواد[۲]، رئوف عماره کربه[۲]، فوزی بولکنافت [۳]، و سلیمه برشی [۱]

۱. آزمایشگاه بیوسیستماتیک و بوم‌شناسی بندپایان، دانشکده علوم طبیعی و زیستی، دانشگاه کنستانتین ۱ – برادران منتوری، جاده عین‌البی، کنستانتین ۲۵۰۱۷، الجزایر

۲. دانشگاه محمد البشیر الابراهیمی، ۳۴۰۳۰، الانصر – برج بوعریریج، الجزایر

۳. دانشگاه سکیکده، سکیکده، الجزایر

**چکیده:** یک بررسی میدانی در مناطق الحضنه (استان مسیله) و البیبان (استان برج بوعریریج) در شمال شرقی الجزایر بین سال‌های ۲۰۰۹ و ۲۰۱۵ انجام شد و با پژوهش‌های میدانی و آزمایشگاهی تکمیلی از سال ۲۰۱۷ تا ۲۰۱۹ کامل گردید. تله‌های چسبان که در پناهگاه‌های حیوانات اهلی در ۳۳ ایستگاه قرار داده شده بودند، در مجموع منجر به تله افتادن ۱۰۷۸۲ نمونه پشه‌ی خاکی متعلق به ۱۴ گونه از ۲ جنس *Phlebotomus* (۸گونه) و *Sergentomyia* (۶ گونه) شدند که ۶۶/۶ از فون ثبت‌شده ی الجزایر را شامل می‌شوند. تنوع زیستی با استفاده از شاخص‌های شانون–ویور(H′) ، سیمپسون (D) و یکنواختی (E) ارزیابی شد. آزمونHutcheson t-test نشان داد که تنوع در منطقه الحضنه (۲/۳۴ = H′) به‌طور معنی‌داری بیشتر از منطقه البیبان است(H' = 1.98) این نتیجه، با وجود غنای گونه‌ای کمتر بیانگر یکنواختی بیشتر در توزیع فراوانی گونه‌ها در الحضنه است. تجزیه و تحلیل تحلیل کای–اسکوئر تفاوت‌های بسیار معنی‌داری را در ترکیب گونه‌ای بین دومنطقه نشان داد. آزمون Mann-Whitney U نشان دادند که فراوانی گونه‌های *Phlebotomus perniciosus*، *P. papatasi* و *P. longicuspis* تفاوت معنی‌داری با یکدیگر دارد. تحلیل همبستگی اسپیرمن با متغیر ارتفاع، ترجیحات زیستگاه الگوهای متفاوتی از وابستگی به زیستگاه را آشکار کرد به طوری که گونه *P. perniciosus* با ارتفاعات بالاتر مرتبط بود، در حالی که گونه *P. papatasi* با مناطق استپی کم‌ارتفاع‌تر ارتباط داشت. برآوردهای Chao1 نیز کامل بودنِ تقریبی نمونه برداری‌ها را تأیید کردد. سه گونه ناقل مهم لیشمانیوز، شامل *P. papatasi*، *P. sergenti*و *P. perniciosus*، در هر دو منطقه به‌صورت سمپاتریک (هم‌مکان) مشاهده شدند که بیانگر خطر بالقوه انتقال اشکال مختلف لیشمانیوز است.

**کلمات کلیدی:** تنوع پشه‌های خاکی، مناطق استپی، ناقلان لیشمانیوز، شمال شرقی الجزایر.

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**نویسنده مسئول:** طاهر سیاح

**ایمیل:** t.sayah@univ-bba.dz

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