



Seasonal dynamics of a population of *Phlebotomus (Larrousius) perfiliewi* Parrot, 1930 (Diptera: Psychodidae) in North-Eastern Romania

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Abstract

Sand flies were collected in a location from Romania in order to estimate their abundance and seasonal variation in correlation with environmental and anthropic factors. From May to October 2017, eight premises with different animal species were sampled for sand flies in a household from Fundătura village, Vaslui County, in North-Eastern Romania. Animal-related data, shelter-related data, and climatic parameters were recorded. All ($n = 150$) collected sand flies were *Phlebotomus perfiliewi*. A mono-modal type of abundance trend has been recorded (a single peak at the beginning of August). The first day of capture was in mid-July. The total number of females during the peak season was significantly higher than the total number of males. The highest percentage of males was recorded at the beginning and at the end of the sand fly activity. Only the traps placed in the poultry enclosure built from clay and wood were positive. A strong positive correlation was recorded between the total number of collected sand flies and the minimum and the maximum temperature. The analysis of the climatic data shows that the first presence of sand flies was registered only after the average minimum temperature for the previous 7 days was above 15 °C.

Keywords Seasonal dynamic · Abundance · Sand flies · *Phlebotomus perfiliewi* · *Leishmania infantum* · Romania

Background

Phlebotomine sand flies (Diptera: Psychodidae) are the principal hematophagous insects confirmed to be competent vectors for the protozoan parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae), responsible for different

forms of leishmaniasis in mammals, including humans (Killick-Kendrick 1999). In Europe and especially in the Mediterranean region, *Leishmania infantum* is the main causative agent of human visceral leishmaniasis (VL) and canine leishmaniasis (CanL). The main vectors in Europe are *Phlebotomus ariasi*, *P. perniciosus*, *P. perfiliewi*,

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P. neglectus, *P. balcanicus*, *P. kandelakii*, *P. langeroni*, and *P. tobbi* (Maroli et al. 2013). *Leishmania infantum* has been also reported to be spreading into parts of Central Europe where autochthonous cases of animal leishmaniasis have been reported: Germany (Bogdan et al. 2001), northern Italy (Maroli et al. 2008), Hungary (Tánczos et al. 2012), or Romania (Mircean et al. 2014).

Romania is a country with a temperate continental climate and with Mediterranean climatic influences in the southern regions. It was traditionally regarded as a non-endemic country for VL and CanL, only with historical sporadic autochthonous human and canine cases described (Manicattide 1919-1920; Mihăilescu and Niciloff 1934; Copăceanu et al. 1955; Minculescu et al. 1955; Lupaşcu et al. 1957; Lupaşcu et al. 1968). However, recent reports of autochthonous VL (Gogoşe et al. 2013) and CanL (Mircean et al. 2014) have been described. Moreover, a serological survey on CanL in Romania indicated the presence of a CanL focus, with a low, but alarming prevalence for a non-endemic country (Dumitrache et al. 2016). Recent imported cases of CanL in foci and areas with possible presence of vectors raised the awareness on this zoonosis in Romania (Pavel et al. 2017; Tanase et al. 2018). Furthermore, the high number of the Romanian workers performing seasonal agricultural labor in Mediterranean countries has been linked to several imported VL in the country (Neghina et al. 2009). Eight species of sand flies were identified so far in Romania: *P. papatasi*, *P. alexandri*, *P. sergenti*, *P. longiductus*, *P. balcanicus*, *P. neglectus*, *P. perfiliewi*, and *Sergentomyia minuta* (Dancesco 2008). For all species, a dataset of geographical coordinates and distribution maps based on historical data was recently published (Dumitrache et al. 2016). From the eight species present in Romania, only three (*P. neglectus*, *P. perfiliewi*, and *P. balcanicus*) are competent vectors for *L. infantum* (Maroli et al. 2013).

The risk of VL and CanL caused by *L. infantum* in endemic countries is influenced by the density of the vector populations (Risueño et al. 2017). Sand flies have a seasonal activity and their density can be influenced by a series of factors such as geographical distribution, temperature, and other climatic parameters or microhabitat particularities (Alten et al. 2016). The seasonal activity of sand flies was studied in most Mediterranean countries (i.e., Portugal, Spain, France, Italy, Greece, Cyprus, Turkey, and Palestinian Territories) where one to three generations of adults have been recorded per season (Alten et al. 2016; González et al. 2017; Sawalha et al. 2017). However, no seasonal dynamic studies from countries at the northern border of sand fly distribution with temperate continental climate are available (<https://ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/phlebotomine-maps>).

In the actual context of the apparent re-emergence of VL and CanL in Romania as well as the increased number of imported cases, the present study was designed to understand the sand fly abundance and seasonal dynamics in a non-Mediterranean country situated at the northern border of sand fly distribution.

Materials and methods

Study area, microhabitat, and insect collection

The study was conducted under the frame of VectorNet project, using a standardized protocol (ECDC and EFSA 2018). The study was performed in the village of Fundătura (46.794333 N, 28.030333 E), Vaslui County, Romania, where a population of sand flies has been found during previous field studies. Fundătura is a small village with a permanent population of 475 inhabitants (Romanian National Institute of Statistics 2013) and has an agricultural-based local economy. It has a typical temperate continental climate with eastern influences, high thermal amplitudes, and non-uniform precipitations. The average annual temperature is around 9 °C and the average annual rainfall between 450 and 700 mm (based on data provided by the Romanian National Meteorological Administration). Sand fly abundance was recorded in several animal premises from a single traditional Romanian household. The equipment used consisted in CDC (Centre for Disease Control and prevention) miniature light traps (Trappola per Monittoraggio Zanzare, IMT Original 2002, Italy). Four traps were placed in the poultry and pigeon house (clay and wood structure), a trap in the cow stable (brick structure), a trap in the horse stable (brick structure), a trap in the pig shed (brick structure), and a trap in the rabbit enclosure (brick structure). The household included the living house located close to the animal shelters and an interior yard with shelters on three sides and a fence on the fourth side (Fig. 1). Some shelters in the area are built in a traditional way, with a composition of clay and dry straw on a wood structure (Fig. 2), while some others were newly built using bricks. Organic matter and animal dejections were abundant near the animal shelters. Each premise was sampled nine times for two consecutive nights at each sampling, at 19-day intervals, between 10th of May and 26th of October 2017. The total number of light trap/days placed in the study was 144 (1 trap × 8 premises × 2 consecutive night × 9 times). The traps were set in and around the animal shelters, on the very same place for the entire study, close to the walls, at a 1.5 m height from the ground. After each trapping night, the insects were collected, stored in tubes with 70% ethanol, and taken to the laboratory for species identification.

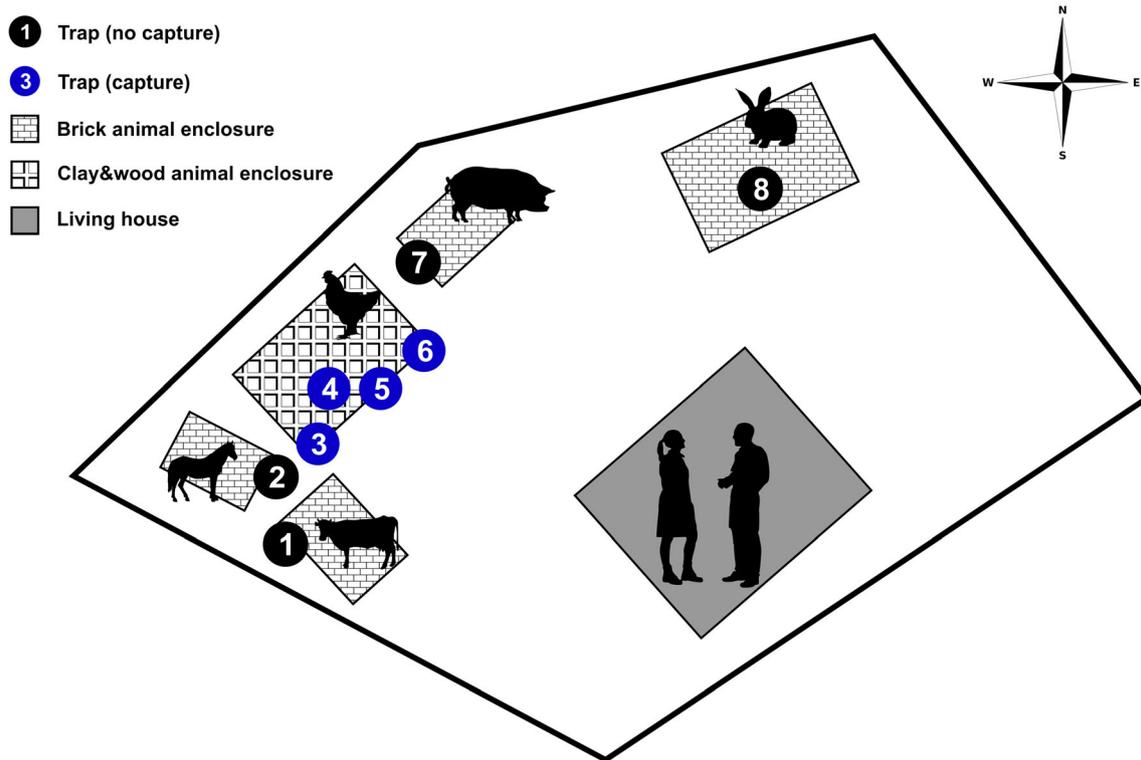


Fig. 1 Schematic representation of the positive and negative traps, animal species, and materials used in the construction of the enclosures

Species identification

Sand flies were separated from the other insects and morphologically identified using entomological keys (Lewis 1982). The males were identified according to the specific features in the aedeagus, stylopodite, and coxopodite, and the females were identified based on the pharynx and spermatheca.

Fig. 2 Actual photo of the shelters in the study area, built in a traditional way, with a composition of clay and dry straw on a wood structure



Environmental data collection

The minimum, maximum, average daily temperatures, average relative humidity, wind speed, and precipitations for the study locality were collected from the Romanian National Meteorological Administration (RNMA) for each day of the sampling period (see Online Resource 1).

Statistical analysis

First, the distribution of data was assessed using D’Agostino–Pearson normality test. Then, the correlation between the number of specimens collected and temperature (min, max, and average), percentage of humidity, precipitations, wind speed, sampling period, and host/house type were determined using Pearson or Spearman’s rank correlation tests. The strength of correlation was established based on absolute value of $r_{(s)}$ coefficient, as follows: 0.00–0.19 “very weak” correlation; 0.20–0.39 “weak” correlation; 0.40–0.59 “moderate” correlation; 0.60–0.79 “strong” correlation; 0.80–1.0 “very strong” correlation. Multiple regression analysis was performed to identify the independent variables that influence/can predict the presence/absence of sand flies. Independent samples t test was used to compare the number of collected sand flies by gender and sampling period. The significance level was set at a value of $p \leq 0.05$. The statistic was performed with MedCalc software.

Results

All ($n = 150$) collected sand flies were *Phlebotomus perfiliewi*. The highest abundance was recorded at the beginning of August, accounting for 71.3% (107/150) of all captured sand flies (Fig. 3). The first day with positive traps was 12 July 2017 and the last day 24 August 2017 (Table 1). Three sampling periods out of nine were positive for sand fly collection, sampling 4 (S4) (12 and 13 July 2017), sampling 5 (S5) (2 and 3 August 2017), and sampling 6 (S6) (23 and 24 August 2017) (Table 1). A statistical correlation between the total number of collected sand flies and the sampling period

was recorded. When comparing the total number of collected sand flies per sampling to S4 and S6, respectively, S5 had the highest number of collected specimens (S5 vs. S4 $p = 0.12$, and S5 vs. S6 $p = 0.05$). No statistical difference between S4 and S6 was recorded (S4 vs. S6 $p = 0.31$). From the total number of collected sand flies, 18% were males and 82% were females ($p = 0.0001$), statistically significant only for S5 (90.7% 97/107 females, and 9.3% 10/107 males; $p = 0.05$) (Table 1). The highest percentage of males (48.1%; 13/27) was found in the first night of insect collection, and the lowest (9.3%) at the end of the season ($p = 0.03$). From the total number of females, 20.3% were blood-fed, with the highest percentage (84.0%; 21/25) at the beginning of August ($p = 0.001$). Raw data and details on number of specimens, gender, and blood-fed females for each positive trap and each positive collecting day are presented (see Online Resource 2). Only the four traps placed in the poultry enclosure built from clay and wood were positive, with a very strong statistical correlation between the number of sand flies collected and the host (poultry)/material used to build the shelters ($r_s = 0.99$, $p = 0.0001$) (Fig. 4a). Climatic data details for the nights with sand fly collection are provided in Online Resource 1. The statistical analysis of the climatic data showed a strong correlation between the total number of collected sand flies with the minimum temperature ($r_s = 0.62$, $p = 0.007$, 95% CI 0.21–0.84) (Fig. 4b) and maximum temperature ($r_s = 0.67$, $p = 0.003$, 95% CI 0.30–0.87) (Fig. 4c), and a medium correlation with the average temperature ($r_s = 0.548$, $p = 0.018418$) (Fig. 4d). The average daily temperature for the days with sand fly collection was between 16.3 ± 5.1 °C and 31.0 ± 6.1 °C, while for the days with no sand fly collection, it was between 11.4 ± 6.2 °C and 24.4 ± 7.3 °C (Table 2). The total number of collected sand flies was negatively correlated with increased

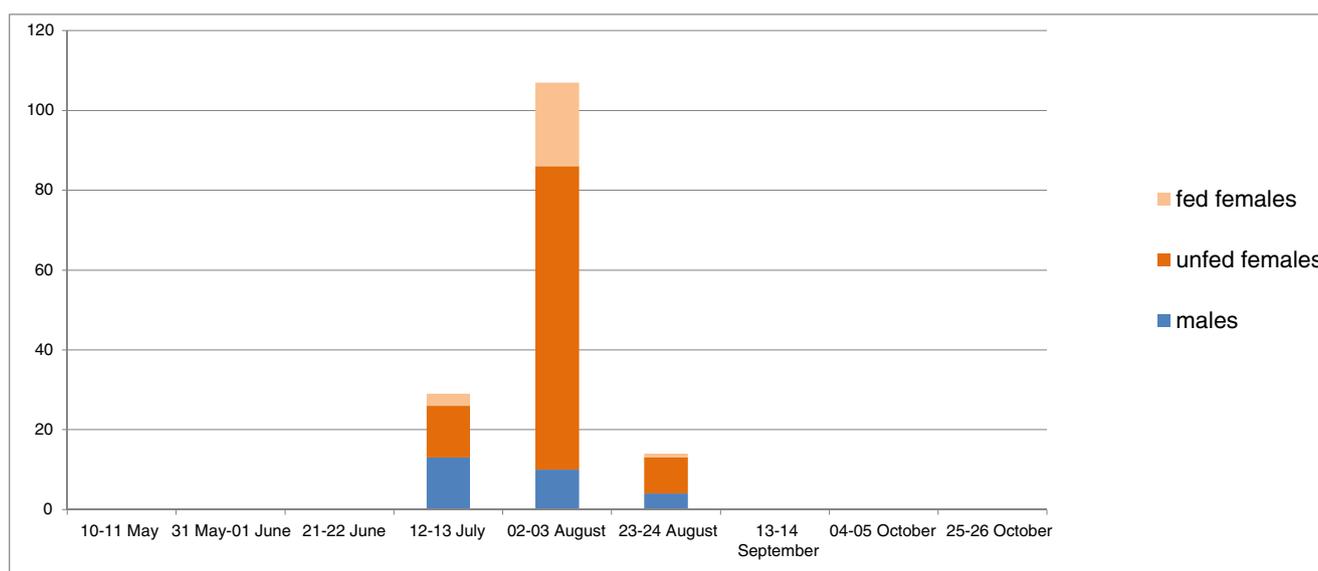


Fig. 3 The recorded abundance in the study (number of collected sand flies/trapping nights)

Table 1 Number of *P. perfiliewi* trapped, by date, gender and feeding status

Sand fly category	Sampling 4 (S4)		Sampling 5 (S5)		Sampling 6 (S6)		Total
	12 July	13 July	02 August	03 August	23 August	24 August	
Males (%)	8 (42.1%) 13 (44.8%) ^a	5 (50.0%)	5 (8.3%) 10 (9.3%)	5 (10.6%)	2 (28.6%) 4 (28.6%) ^a	2 (28.6%)	27 (18.0%)
Females (%)	11 (57.9%) 16 (55.2%) ^a	5 (50.0%)	55 (91.7%) 97 (90.7%)	42 (89.4%)	5 (71.4%) 10 (71.4%) ^a	5 (71.4%)	123 (82.0%)
Unfed females (%)	8 (72.7%) 13 (81.3%) ^a	5 (100%)	36 (65.5%) 76 (78.4%)	40 (95.2%)	5 (100%) 9 (90.0%) ^a	4 (80.0%)	98 (79.7%)
Fed females (%)	3 (27.3%) 3 (18.7%) ^a	0 (0%)	19 (34.5%) 21 (21.6%)	2 (4.8%)	0 (0%) 1 (10.0%) ^a	1 (20.0%)	25 (20.3%)
Total sand flies (%)	19 (12.7%) 29 (19.3%) ^{a,b}	10 (6.7%)	60 (40.0%) 107 (71.3%) ^a	47 (31.3%)	7 (4.7%) 14 (9.3%) ^b	7 (4.7%)	150

Values with no common superscript in a column were significantly different ($p < 0.05$)

values of the average relative humidity RH% ($r_s = -0.450$, $p = 0.061118$) (Fig. 4e). A weak statistical negative correlation was registered between the total numbers of sand flies and the precipitations ($r_s = 0.290$, $p = 0.243018$) (Fig. 4f) and wind speed ($r_s = -0.211$, $p = 0.399918$) (Fig. 4g). The average humidity for the days with collected sand flies was between RH% 45.5 ± 7.3 and 63 ± 9.9 , while for the days with no sand fly collection it was between RH% 53.5 ± 3.5 and RH% 61.9 ± 4.8 (Table 2). The average wind speed (as provided by RNMA) for the days with collected sand flies was between 10.5 ± 2.4 km/h and 22 ± 2.8 km/h, while for the days with no sand fly collection, it was between 11.3 ± 1.7 km/h and 17.1 ± 1.8 km/h (Table 2).

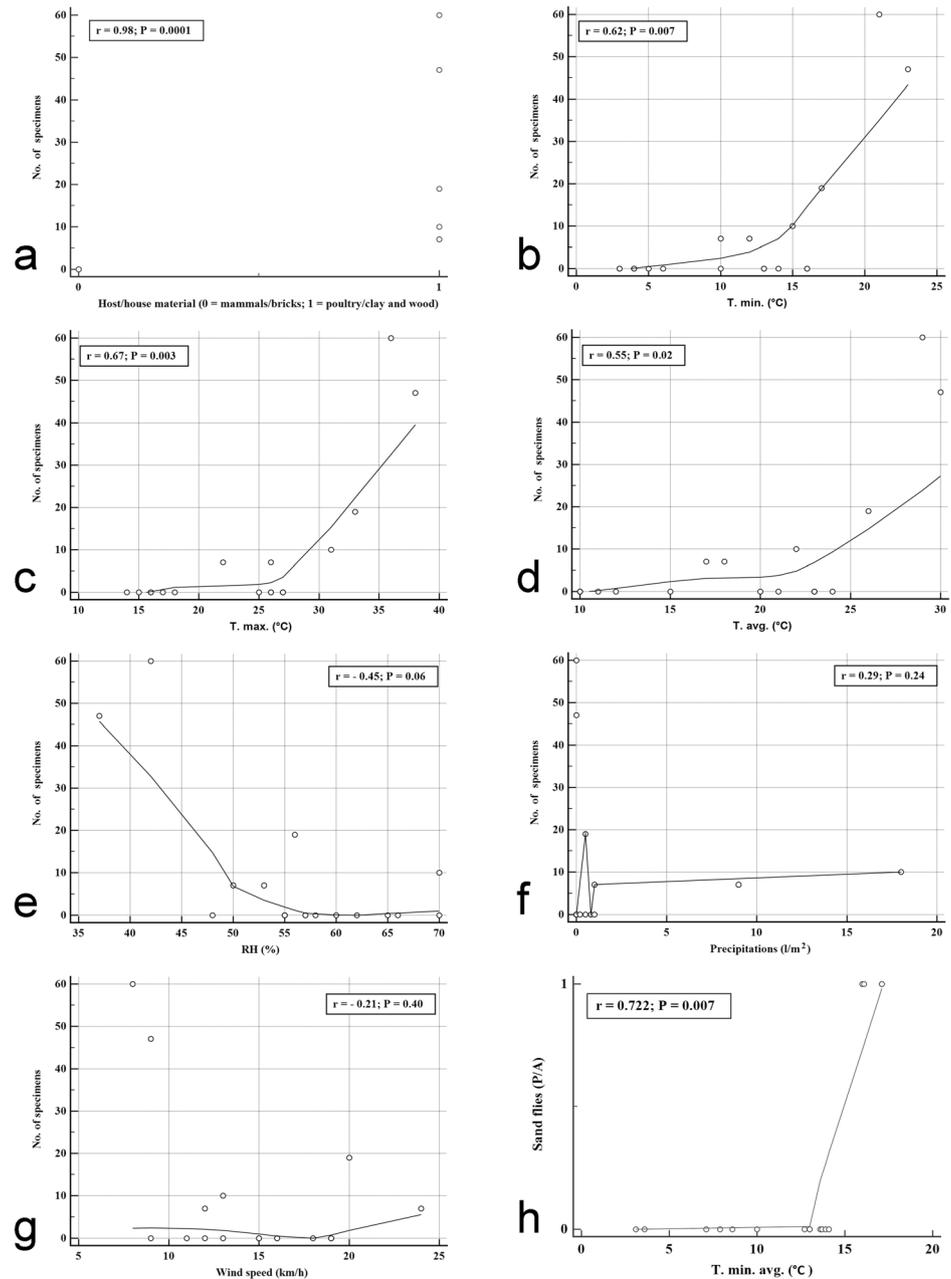
The analysis of the climatic data shows that the first presence of sand flies was registered only after the average minimum temperature for the previous 7 days was above 15°C ($p = 0.0322$) (Fig. 4h). When simultaneously compared to multiple variables (climatic and environmental), a statistical significance of the sand fly presence was observed ($p = 0.0004$) (Table 3).

Discussion

The epidemiology of sand fly-borne diseases is highly influenced by many factors, including the sand fly distribution, seasonal activity, density, and number of generations per year. The present study is the first to evaluate the seasonal dynamics of sand flies in a non-Mediterranean European country, situated at the northern border of sand fly distribution. In the Mediterranean basin, endemic for both VL and CanL, studies evaluating the seasonal dynamics of the phlebotomine vectors are available in France (Rioux et al. 2013; Prudhomme et al. 2015; Alten et al. 2016), Italy (Maroli et al. 1977; Rossi et al. 2008; Tarallo et al. 2010; Lisi et al. 2014; Alten et al. 2016), Spain (Gálvez et al. 2010; Risueño et al. 2017; Alten et al.

2016; González et al. 2017), Greece (Ivovic et al. 2007; Chaskopoulou et al. 2016; Alten et al. 2016; Boutsini et al. 2017), Portugal (Maia et al. 2009; Alten et al. 2016), Turkey (Volf et al. 2002; Kasap et al. 2009; Alten et al. 2016), Cyprus (Alten et al. 2016), the Palestinian Territories (Sawalha et al. 2017), and Georgia (Alten et al. 2016) (Table 4). Across its Mediterranean distribution range, the seasonal dynamics of *P. perfiliewi* was studied in Greece (Ivovic et al. 2007; Chaskopoulou et al. 2016), Italy (Maroli and Bettini 1977; Rossi et al. 2008; Tarallo et al. 2010; Lisi et al. 2014), Turkey (Volf et al. 2002; Kasap et al. 2009), and the Palestinian Territories (Sawalha et al. 2017) over a period of one to six collecting seasons (Table 4). The number of peak months of sand fly activity is associated with the number of generations of adults (Alten et al. 2016). The mono-modal trend (single peak occurring in July–August) is one of the four trends described so far for sand flies (Alten et al. 2016), and the one anticipated to be present in Romania, based on the degree-day developmental requirements described for *P. papatasi* in Turkey (Kasap and Alten 2005). These developmental requirements were confirmed for average daily temperatures for an entire year in four macro-regions in Romania before the study was performed (data not shown) in order to estimate the best interval for the trapping period, the possible positive months for sand fly activity, and the number of generations of adults/collecting season. The present study confirms the mono-modal abundance trend for *P. perfiliewi* in Romania. A highly significant correlation was described between latitude, altitude, annual average temperature, the first date of sand fly collection, and the type of the abundance trend (Alten et al. 2016). *Phlebotomus ariasi* (France), *P. kandelakii* (Georgia), and *P. balcanicus* (Georgia) displayed a mono-modal trend as *P. perfiliewi* in the present study. The recorded latitudes for these species were situated between 41.700278°N (Georgia), 43.973056°N (France), and 46.794333°N (current study in Romania). *Phlebotomus perfiliewi* displayed a mono-

Fig. 4 a–h The results of the statistical analysis



modal trend in all the locations where its seasonal abundance was studied (Greece, Italy, and Palestinian Territories) (Table 4). The mono-modal trend was present at different latitudes, even though a highly significant correlation between a latitude decrease and the increase in number of vector density peaks was described for some other sand fly species (Alten et al. 2016). For example, *P. perniciosus*, a sympatric species for *P. perfiliewi* across most of their distribution range, displayed a confluent bi-modal trend (two confluent peaks) in Spain, Italy, and Central Portugal, at higher latitudes, while in Southern Portugal, it displayed a distinctive bi-modal trend (two distinctive peaks) (Alten et al. 2016). *Phlebotomus perniciosus* displayed a tri-modal trend (three distinctive

peaks) in Italy (Lisi et al. 2014). Similar differences were observed for *P. tobbi*, which has a tri-modal abundance trend in southern Cyprus, and a bi-modal abundance trend in Turkey (Alten et al. 2016).

The variations in the distribution, diversity, and abundance trends of the sand fly species were correlated with climatic factors (temperature and precipitations) and environmental factors (geographical barriers, habitat, abundance, and distribution of the vertebrate hosts) in most of the available studies (Volf et al. 2002; Kasap et al. 2009; Ivovic et al. 2007; Simsek et al. 2007; Tarallo et al. 2010; Alten et al. 2016). The climate, as a result of the biotic and abiotic properties of one environment, correlated with the altitudinal gradient and the latitude,

Table 2 Daily average climatic values (min, max) during the trapping dates

Days with	Average temperature (°C)		Wind (km/h)		Relative humidity (RH%)	
	Min	Max	Min	Max	Min	Max
Uncollected sand flies	11.4 ± 6.2	24.4 ± 7.3	11.3 ± 1.7	17.1 ± 1.8	53.5 ± 3.5	61.9 ± 4.8
Collected sand flies	16.3 ± 5.1	31.0 ± 6.1	10.5 ± 2.4	22 ± 2.8	45.5 ± 7.3	63 ± 9.9
Uncollected males	11.4 ± 6.2	24.4 ± 7.3	12.5 ± 2.3	17.1 ± 1.8	53.5 ± 3.5	61.9 ± 4.8
Collected males	16.3 ± 5.1	31.0 ± 6.7	10.5 ± 2.4	22 ± 2.8	45.5 ± 7.3	63 ± 9.9
Uncollected females	11.4 ± 6.2	24.4 ± 7.3	10.5 ± 2.4	22 ± 2.8	53.5 ± 3.5	61.9 ± 4.8
Collected females	16.3 ± 5.1	31.0 ± 6.7	11.3 ± 1.7	17.1 ± 1.8	45.5 ± 7.3	63 ± 9.9
Uncollected blood-fed females	11.4 ± 6.2	24.4 ± 7.3	10.5 ± 2.4	22 ± 2.8	53.5 ± 3.5	61.9 ± 4.8
Collected blood-fed females	16.3 ± 5.1	31.0 ± 6.7	10.5 ± 2.4	22 ± 2.8	45.5 ± 7.3	63 ± 9.9

are the most important factors that influence the sand fly life span (development period) in one territory (Belen and Alten 2005; Simsek et al. 2007). The non-optimal environmental conditions, limited feeding sources, or host population densities do not affect only the development period of sand flies, but also their phenotype and behaviour (Belen et al. 2004; Oguz et al. 2017). Only one sand fly species was recorded in our study, compared to a higher diversity in most other countries (Table 4). This can be explained by a series of factors: (1) the climate is temperate continental with Baltic and eastern influences in north and North-Eastern Romania, with annual average temperatures around 9 °C, colder than the southern region of Romania with Mediterranean climatic influences (Ielenicz and Pătru 2005) where a higher sand fly species diversity was recorded (Dancesco 2008); (2) between 1958 and 1964, insecticides were widely used in the Malaria eradication programs (Dancesco 2008); (3) in the last decades, the sand fly species composition changed due to environmental, demographic, and climate changes that negatively impacted the sand fly habitats (Maroli et al. 2013). A positive correlation of the sand fly diversity and altitude, with a relative high number of sand fly species present around 900-m altitude, was described (Simsek et al. 2007). In our study, conducted at 198-m altitude, the diversity of the sand fly species was very low,

with only one species present, but compared to other studies (Simsek et al. 2007), this happens at a higher latitude. In the present study, the activity of *P. perfiliewi* started in July and ended in August. In other countries, this sand fly species started its activity in April, May, or June, according to the locality and year of study (Table 4). In Italy, for example, the species was active at mean temperatures situated between 27.09 and 28.02 °C at 372 m above sea level (Tarallo et al. 2010). In Greece, the species was more abundant in the continental part of the country, more related to the humid and sub-humid climate, than in the islands, where a distinctly hot and dry Mediterranean climate is present (Ivovic et al. 2007). In Greece, the activity of *P. perfiliewi* ended also in August (Ivovic et al. 2007), but other studies recorded this species as late as October in Greece (Chaskopoulou et al. 2016) or November and December in Italy (Tarallo et al. 2010; Lisi et al. 2014). September and October were the last months of *P. perfiliewi* activity in Turkey (Volf et al. 2002; Kasap et al. 2009), and November in Palestinian Territories (Sawalha et al. 2017). No significant correlation was found between latitude/mean annual temperature of the sites and the end of the phlebotomine sand flies activity (Alten et al. 2016).

In our study, the peak season of *P. perfiliewi* was recorded in July–August, similar to one study in Italy (Tarallo et al.

Table 3 Multiple regression analysis of independent variables that influence/can predicts the presence/absence of sand flies

		Coefficient	Std. error	t	P	r_{partial}	$r_{\text{semipartial}}$
Independent variables	(Constant)	2.8889					
	T°C (min/daily)	0.09478	0.03812	2.486	0.0322	0.6181	0.2559
	T°C (max/daily)	−0.07516	0.03676	−2.045	0.0681	−0.5429	0.2104
	T°C (average/daily)	−0.01676	0.04503	−0.372	0.7175	−0.1169	0.03831
	RH% (average/daily)	−0.02196	0.007068	−3.107	0.0111	−0.7008	0.3198
	Precipitations (l/m ²)	−0.02480	0.01179	−2.103	0.0618	−0.5537	0.2164
	Wind speed (km/h)	−0.03756	0.01304	−2.880	0.0164	−0.6734	0.2965
	Host/Material	0.7641	0.2043	3.740	0.0038	0.7636	0.3850
Analysis of variables		12.055	–	–	0.0004	0.3974	

Table 4 Phlebotomine species described in abundance studies in the Old World (literature review)

	Sampling seasons (period)	Number of species collected	No. of generations/year	Species ^a	First date of collection	Last date of collection	Peak month	References	
Cyprus	2 (2012–2013)	3	Not specified	<i>P. galilaeus</i>	May	October	Not specified	(Alten et al. 2016)	
					May	October	Not specified		
France	1 (1981)	3	3	<i>P. tobbi</i>	May	October	June, August, September	(Rioux et al. 2013)	
					May	October	July and October		
					May	October	June–July		
					May	October	Not specified		
					May	October	Not specified		
					May	October	July		(Prudhomme et al. 2015)
					May	October	August		
					May	October	July		
					May	October	July		
					Georgia	3 (2011–2013)	3		1
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
May	October	Not specified							
Greece	6 (1999–2004)	10	1	<i>P. ariasi</i>				June	
					June	August	June–August		
					June	August	June–August		
					June	August	June–August		
					June	August	June–August		
					June	August	June–August		
					June	August	June–August		
					June	August	June–August		
					June	August	June–August		
					June	August	June–August		
Greece	3 (2011–2013)	3	2	<i>P. neglectus</i>	May	October	May–June, August–September	(Alten et al. 2016)	
					May	October	Not specified		
					May	October	Not specified		
					May	October	Not specified		
					May	October	Not specified		
					May	October	Not specified		
Greece	1 (2011)	6	1	<i>P. papatasi</i>	May	October	August–September		
					May	October	August–September		
					May	October	August–September		
					May	October	August–September		
					May	October	August–September		
					May	October	August–September		

Table 4 (continued)

Sampling seasons (period)	Number of species collected	No. of generations/year	Species ^a	First date of collection	Last date of collection	Peak month	References
		1	<i>P. perfliewi</i>	May	October	August–September	(Chaskopoulou et al. 2016)
		1	<i>P. simici</i>	May	October	August–September	
		1	<i>P. tobbi</i>	May	October	August–September	
		1	<i>S. dentata</i>	May	October	August–September	
		1	<i>S. minuta</i>	May	October	August–September	
4 (exact years of the study not specified)	7	Not specified	<i>P. alexandri</i>	June	September	Not specified	(Boutsini et al. 2017)
		1	<i>P. neglectus</i>	June	September	August	
		1	<i>P. papatasi</i>	June	September	August	
		1	<i>P. simici</i>	June	September	September	
		Not specified	<i>P. similis</i>	June	September	Not specified	
		3	<i>P. tobbi</i>	June	September	Not specified	
		3	<i>S. minuta</i>	June	September	June, August, September	
1 (1975)	5	1	<i>P. mascittii</i>	June	September	June, August, September	Maroli and Bettini (1977)
		1	<i>P. papatasi</i>	June	November	August–September	
		1	<i>P. perfliewi</i>	June	November	August–September	
		2	<i>P. perniciosus</i>	June	November	August–September	
		1	<i>S. minuta</i>	June	November	July, August–September	
2 (2002–2003)	4	2	<i>P. mascittii</i>	May	November	August–September	(Rossi et al. 2008)
		2	<i>P. papatasi</i>	May	November	June, August	
		2	<i>P. perniciosus</i>	May	November	June, August	
		2	<i>S. minuta</i>	May	November	June, August	
2 (2008–2009)	5	1	<i>P. neglectus</i>	June	October	July–August	(Tarallo et al. 2010)
		1	<i>P. papatasi</i> (2008)	June	November	July–August	
		1	<i>P. perfliewi</i> (2008)	May	November	July–August	
		1	<i>P. perniciosus</i>	May	November	July–August	
		1	<i>S. minuta</i>	May	November	July–August	
2 (2006 and 2013)	6	Not specified	<i>P. mascittii</i>	April	October	July–August	(Lisi et al. 2014)
		Not specified	<i>P. neglectus</i>	April	December	Not specified	
		Not specified	<i>P. perfliewi</i>	April	December	Not specified	
		3	<i>P. perniciosus</i> (2006)	April	December	Not specified	
		2	<i>P. perniciosus</i> (2013)	April	December	June, August, September	
		1	<i>P. sergenti</i>	April	December	June, September	
		Not specified	<i>S. minuta</i>	April	December	July	
2 (2011–2012)	1	2	<i>P. perniciosus</i>	May	October	Not specified	(Alten et al. 2016)
1 (2011)	22	1	<i>P. alexandri</i>	May	November	June–September	

Table 4 (continued)

	Sampling seasons (period)	Number of species collected	No. of generations/year	Species ^a	First date of collection	Last date of collection	Peak month	References
Palestinian Territories			1	<i>P. canaaniticus</i>	May	November	August	(Sawalha et al. 2017)
			1	<i>P. halepensis</i>	May	November	October	
			1	<i>P. jacusieli</i>	May	November	August	
			1	<i>P. kazeruni</i>	May	November	June	
			1	<i>P. major</i>	May	November	June	
			1	<i>P. neglectus</i>	May	November	October	
			2	<i>P. papatasi</i>	May	November	June, October	
			1	<i>P. perfluvii</i>	May	November	October	
			1	<i>P. saltiae</i>	May	November	June	
			2	<i>P. sergenti</i>	May	November	July, October	
			2	<i>P. syriacus</i>	May	November	July, October	
			1	<i>P. tobbi</i>	May	November	August–September	
			1	<i>S. adleri</i>	May	November	June	
			1	<i>S. Africana</i>	May	November	July	
			1	<i>S. antennata</i>	May	November	July	
			1	<i>S. christophersi</i>	May	November	August	
			1	<i>S. dentate</i>	May	November	August	
			1	<i>S. fallax</i>	May	November	August	
			1	<i>S. taizi</i>	May	November	June–August	
			1	<i>S. theodori</i>	May	November	June	
		1	<i>S. tiberiadis</i>	May	November	September		
Portugal	1 (2006)	5	1	<i>P. ariasi</i>	May	November	July	(Maia et al. 2009)
			1	<i>P. papatasi</i>	May	November	July	
			1	<i>P. permiciosus</i>	May	November	July	
			1	<i>P. sergenti</i>	May	November	July	
			1	<i>S. minuta</i>	May	November	July	
			Not specified	<i>P. ariasi</i>	May	October	Not specified	(Allen et al. 2016)
	3 (2011–2013)	3	2	<i>P. permiciosus</i>	May	October	May–June, August–September	
			Not specified	<i>P. sergenti</i>	May	October	Not specified	
			1	<i>P. ariasi</i>	May	November	August	(Gálvez et al. 2010)
			Not specified	<i>P. papatasi</i>	May	November	Not specified	
Spain	1 (2008)	5	2	<i>P. permiciosus</i>	May	November	July, September	
			Not specified	<i>P. sergenti</i>	May	November	Not specified	
			Not specified	<i>S. minuta</i>	May	November	Not specified	
			Not specified	<i>P. papatasi</i>	May	October	Not specified	
			3	<i>P. papatasi</i>	May	October	Not specified	
	2 (2012–2013)	3	Not specified	<i>P. papatasi</i>	May	October	Not specified	(Allen et al. 2016)

Table 4 (continued)

	Sampling seasons (period)	Number of species collected	No. of generations/year	Species ^a	First date of collection	Last date of collection	Peak month	References
			2	<i>P. permiciosus</i>	May	October	June, September	
			Not specified	<i>P. sergenti</i>	May	October	Not specified	
	3 (2012–2014)	4	Not specified	<i>P. papatasi</i>	June	October	Not specified	(González et al. 2017)
			2	<i>P. permiciosus</i> (2012)	June	October	June, August	
			1	<i>P. permiciosus</i> (2013)	June	October	July	
			1	<i>P. permiciosus</i> (2014)	June	October	September	
			Not specified	<i>P. sergenti</i>	June	October	Not specified	
			Not specified	<i>S. minuta</i>	June	October	Not specified	
	1 (2015)	8	1	<i>P. alexandri</i>	May	October	July	(Risueño et al. 2017)
			1	<i>P. ariasi</i>	May	October	July	
			1	<i>P. chabaudi</i>	May	October	July	
			1	<i>P. longicauspis</i>	May	October	July	
			1	<i>P. papatasi</i>	May	October	July	
			1	<i>P. permiciosus</i>	May	October	July	
			1	<i>P. sergenti</i>	May	October	July	
			1	<i>S. minuta</i>	May	October	July	
Turkey	3 (1997–1999)	10	Not specified	<i>P. alexandri</i>	May	September	Not specified	(Volf et al. 2002)
			Not specified	<i>P. brevis</i>	May	September	Not specified	
			Not specified	<i>P. halepensis</i>	May	September	Not specified	
			Not specified	<i>P. mascitii</i>	May	September	Not specified	
			Not specified	<i>P. neglectus</i>	May	September	Not specified	
			Not specified	<i>P. papatasi</i>	May	September	Not specified	
			Not specified	<i>P. perflitewi</i>	May	September	Not specified	
			Not specified	<i>P. sergenti</i>	May	September	Not specified	
			Not specified	<i>S. adleri</i>	May	September	Not specified	
			Not specified	<i>S. theodori</i>	May	September	Not specified	
	1 (2006)	5	Not specified	<i>P. papatasi</i>	May	October	May	(Kasap et al. 2009)
			Not specified	<i>P. perflitewi galilaeus</i>	June	October	August	
			Not specified	<i>P. sergenti</i>	July	September	September	
			Not specified	<i>P. tobbi</i>	May	October	May, September	
			Not specified	<i>Sergentomyia spp.</i>	May	September	May	
	2 (2011–2012)	9	Not specified	<i>P. alexandri</i>	May	October	Not specified	(Alten et al. 2016)
			Not specified	<i>P. major s.l.</i>	May	October	Not specified	
			Not specified	<i>P. mascitii</i>	May	October	Not specified	
			Not specified	<i>P. neglectus/syriacus</i>	May	October	Not specified	

Table 4 (continued)

Sampling seasons (period)	Number of species collected	No. of generations/year	Species ^a	First date of collection	Last date of collection	Peak month	References
		Not specified	<i>P. papatasi</i>	May	October	Not specified	
		Not specified	<i>P. sergenti</i>	May	October	Not specified	
		Not specified	<i>P. simici</i>	May	October	Not specified	
		2	<i>P. tobbi</i>	May	October	May–June, August–September	
		Not specified	<i>P. transcaucasicus</i>	May	October	Not specified	

^a If the species was found only in certain years of the study interval or if the seasonal activity for a species was different among the years of the study, these years are mentioned in brackets

2010). In other studies, June–August, August–September, or October was the last months of *P. perfiliewi* activity (Table 4). Also, the differences between the beginning, the peak, and the end of the season for the same vector species in different territories are correlated with the higher or lower altitude in that particular region (Alten et al. 2016). The other sand fly species collected along with *P. perfiliewi* in the same localities in different studies showed similarities, but also differences regarding the beginning, end, and peak of the season (Table 4). In Greece (Chaskopoulou et al. 2016) and Turkey (Volf et al. 2002; Kasap et al. 2009), five and nine sand fly species, respectively, started their activity in May. In Italy (Maroli and Bettini 1977; Tarallo et al. 2010; Lisi et al. 2014), other six sand fly species started their activity in April, May, or June, and in the Palestinian Territories (Sawalha et al. 2017), another 21 sand fly species had their beginning of the season in May (Table 4). There are differences between various sympatric sand fly species with regard to their peak season as well (Table 4). The end of the season for most of the other sand fly species collected along with *P. perfiliewi* in Greece (Chaskopoulou et al. 2016), Italy (Maroli and Bettini 1977; Tarallo et al. 2010; Lisi et al. 2014), Palestinian Territories (Sawalha et al. 2017), and Turkey (Volf et al. 2002; Kasap et al. 2009) was in October, November, or December (Table 4). So far, no significant statistical correlation was described between the end of sand fly activity and the latitude and the average annual temperatures from the collecting sites (Alten et al. 2016).

The present abundance study was designed to understand the seasonal dynamics of sand flies in Romania, a country with temperate climate, with sporadic cases of CanL and VL. Understanding the dynamics of a vector species and its interaction with pathogens can increase the awareness on the vector-borne diseases of public health concern. The possible changes in the environment of the sand flies could affect both the vectors and the risk of disease transmission, with a direct impact on human health (Parham et al. 2015; Oguz et al. 2017). Multi-seasonal abundance studies of sand flies performed in the same region can provide valuable data on the potential transmission of *L. infantum*. As the main vectors of leishmaniasis in Europe display density peaks more or less frequently correlated with southern or northern latitudes (Alten et al. 2016), understanding the seasonal dynamics patterns of *P. perfiliewi* in North-Eastern Romania can also predict the potential risk seasons for *L. infantum* transmission.

Conclusions

Our study is the first of its kind conducted in a non-Mediterranean European country with a temperate climate, situated at the northern border of sand fly distribution and with sporadic cases of VL and CanL. All the data suggest that the

activity season of *P. perfiliewi* is shorter in Romania than in the Mediterranean countries, impacting negatively the risk of disease transmission. The peak density of *P. perfiliewi* was at the beginning of August, providing scientific data which can be further used during awareness campaigns for veterinarians and public health professionals. The re-emergence of canine and human leishmaniasis in Romania and the presence of disease foci in the country raise the need for more studies on the distribution, diversity, and seasonality of the vectors but also for epidemiological studies of VL and CanL.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Alten B, Maia C, Afonso MO, Campino L, Jiménez M, González E, Molina R, Bañuls AL, Prudhomme J, Vergnes B, Toty C, Cassan C, Rahola N, Thierry M, Sereno D, Bongiorno G, Bianchi R, Khoury C, Tsirigotakis N, Dokianakis E, Antoniou M, Christodoulou V, Mazeris A, Karakus M, Ozbel Y, Arserim SK, Erisoz Kasap O, Gunay F, Oguz G, Kaynas S, Tsertsvadze N, Tskhvaradze L, Giorgobiani E, Gramiccia M, Volf P, Gradoni L (2016) Seasonal dynamics of phlebotomine sand fly species proven vectors of Mediterranean leishmaniasis caused by *Leishmania infantum*. *PLoS Negl Trop Dis* 10(2):e0004458
- Belen A, Alten B (2005) Variation in life table characteristics among populations of *Phlebotomus papatasi* at different altitudes. *J Vector Ecol* 31:35–44
- Belen A, Alten B, Aytekin A (2004) Altitudinal variation in morphometric and molecular characteristics of *Phlebotomus papatasi* populations. *Med Vet Entomol* 18:343–350
- Bogdan C, Schonian G, Banuls AL, Hide M, Pralong F, Lorenz E, Röllinghoff M, Mertens R (2001) Visceral leishmaniasis in a German child who had never entered a known endemic area: case report and review of the literature. *Clin Infect Dis* 32:302–306
- Boutsini S, Athanasiou LV, Spanakos G, Ntousi D, Dotsika E, Bisia M, Papadopoulos E (2017) Phlebotomine sandflies and factors associated with their abundance in the leishmaniasis endemic area of Attiki, Greece. *Parasitol Res* 117:107–113
- Chaskopoulou A, Giantsis IA, Demir S, Bon MC (2016) Species composition, activity patterns and blood meal analysis of sand fly populations (Diptera: Psychodidae) in the metropolitan region of Thessaloniki, an endemic focus of canine leishmaniasis. *Acta Trop* 158:170–176
- Copăceanu P, Motilică V, Nicolaescu N, Iliescu I (1955) The first case of visceral leishmaniasis in adults in RPR. *Rev Ig Microbiol Epid* 3: 88–90 (In Romanian)
- Dancesco P (2008) Species of sandflies (Diptera: Psychodidae) in Romania, some aspects of their ecology and new capture stations. *Trav Mus Nat Hist Grigore Antipa* 51:185–199 (In French)
- Dumitrache MO, Nachum-Biala Y, Gilad M, Mircean V, Cazan CD, Mihalca AD, Baneth G (2016) The quest for canine leishmaniasis in Romania: the presence of an autochthonous focus with subclinical infections in an area where disease occurred. *Parasit Vectors* 9:297
- European Centre for Disease Prevention and Control; European Food Safety Authority (2018) Field sampling methods for mosquitoes, sandflies, biting midges and ticks – VectorNet project 2014–2018. ECDC and EFSA, Stockholm and Parma
- Gálvez R, Descalzo MA, Miró G, Jiménez MI, Martín O, Dos Santos-Brandao F, Guerrero I, Cubero E, Molina R (2010) Seasonal trends and spatial relations between environmental/meteorological factors and leishmaniasis sand fly vector abundances in Central Spain. *Acta Trop* 115:95–102
- Gogoșe MG, Teodorescu I, Preda C, Ionescu SC (2013) Two case reports on visceral leishmaniasis diagnosed in Romania. *Roum Arch Microbiol Immunol* 72:49–62
- González E, Jiménez M, Hernández S, Martín-Martín I, Molina R (2017) Phlebotomine sand fly survey in the focus of leishmaniasis in Madrid, Spain (2012–2014): seasonal dynamics, *Leishmania infantum* infection rates and blood meal preferences. *Parasit Vectors* 10:368
- Ielenicz M, Pătru I (2005) Physical geography of Romania, Editura Universitară, Bucharest, ISBN 973-7787-37, pp7 (in Romanian)
- Ivovic V, Patakakis M, Tselentis Y, Chaniotis B (2007) Faunistic study of sandflies in Greece. *Med Vet Entomol* 21:121–124
- Kasap OE, Alten B (2005) Laboratory estimation of degree-day developmental requirements of *Phlebotomus papatasi* (Diptera: Psychodidae). *J Vector Ecol* 30:328–333
- Kasap OE, Belen A, Kaynas S, Simsek FM, Biler L, Ata N, Alten B (2009) Activity patterns of sand fly (Diptera: Psychodidae) species and comparative performance of different traps in an endemic cutaneous leishmaniasis focus in Cukurova Plain, Southern Anatolia, Turkey. *Acta Vet Brno* 78:327–335
- Killick-Kendrick R (1999) The biology and control of phlebotomine sand flies. *Clin Dermatol* 17:279–289
- Lewis DJ (1982) A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bullbr Musnat Hist Entomol* 45:171–209
- Lisi O, D’Urso V, Vaccaluzzo V, Bongiorno G, Khoury C, Severini F, Di Muccio T, Gramiccia M, Gradoni L, Maroli M (2014) Persistence of phlebotomine *Leishmania* vectors in urban sites of Catania (Sicily, Italy). *Parasit Vectors* 9:56032
- Lupașcu G, Ciplea A, Gherman I (1957) Contributions to the study of the reservoir of infection in the outbreak of leishmaniasis in Craiova region. *Stud Cercet Inframicrobiol Parzitol* 8:647–651 (In Romanian)
- Lupașcu G, Bossie A, Ciplea A, Costin P (1968) Research on the animal reservoir of *Leishmania*. *Arch Roum Pathol Exp Microbiol* 28:29–35 (In French)
- Maia C, Afonso MO, Neto L, Dionísio L, Campino L (2009) Molecular detection of *Leishmania infantum* in naturally infected *Phlebotomus perniciosus* from Algarve region, Portugal. *J Vector Borne Dis* 46: 268–272
- Manicatide N (1919–1920) Two cases of Kala-Azar observed in Romania. *Bull Sect Sci Acad Roum*:105 (In French)
- Maroli M, Bettini S (1977) Leishmaniasis in Tuscany (Italy): (I) an investigation on phlebotomine sandflies in Grosseto Province. *Trans R Soc Trop Med Hyg* 71:315–321

- Maroli M, Rossi L, Baldelli R, Capelli G, Ferroglio E, Genchi C, Gramiccia M, Mortarino M, Pietrobelli M, Gradoni L (2008) The northward spread of leishmaniasis in Italy: evidence from retrospective and ongoing studies on the canine reservoir and phlebotomine vectors. *Tropical Med Int Health* 13:256–264
- Maroli M, Felicangeli MD, Bichaud L, Charrel RN, Gradoni L (2013) Phlebotomine sandflies and the spreading of leishmaniasis and other diseases of public health concern. *Med Vet Entomol* 27:123–147
- Mihăilescu M, Niciloff D (1934) Two cases of spontaneous canine leishmaniasis in Romania. *Arhiva Vet* 26:43–53 (In Romanian)
- Minculescu M, Bîrzu I, Crețu S, Iovănescu F, Ionescu D, Lupulescu V et al (1955) Reflections on the first outbreak of infantile leishmaniasis identified RPR. *Stud Cercet Inframicrobiol Parazitol III* 6:596–603 (In Romanian)
- Mircean V, Dumitrache MO, Mircean M, Bolfa P, Györke A, Mihălca AD (2014) Autochthonous canine leishmaniasis in Romania: neglected or (re) emerging. *Parasit Vectors* 7:135
- Neghina R, Neghina AM, Merkler C, Marinicu I, Moldovan R, Iacobiciu I (2009) Importation of visceral leishmaniasis in returning Romanian workers from Spain. *Travel Med Infect Dis* 7:35–39
- Oguz G, Kasap OE, Alten B (2017) Wing morphology variations in a natural population of *Phlebotomus tobbi* Adler and Theodor 1930. *J Vector Ecol* 42:223–232
- Parham PE, Waldo J, Christophides GK, Hemming D, Agosto F, Evans KJ, Fefferman N, Gaff H, Gumel A, LaDeau S, Lenhart S, Mickens RE, Naumova EN, Ostfeld RS, Ready PD, Thomas MB, Velasco-Hernandez J, Michael E (2015) Climate, environmental and socio-economic change: weighing up the balance in vector-borne disease transmission. *Philos Trans R Soc Lond Ser B Biol Sci* 370: 20130551
- Pavel G, Timofte D, Mocanu D, Malancus R, Solcan C (2017) Imported leishmaniasis in a dog in a sandfly-populated area in northeastern Romania. *J Vet Diagn Investig* 29:683–685
- Prudhomme J, Rahola N, Toty C, Cassan C, Roiz D, Vergnes B, Thierry M, Rioux JA, Alten B, Sereno D, Bañuls AL (2015) Ecology and spatiotemporal dynamics of sandflies in the Mediterranean Languedoc region (Roquedur area, Gard, France). *Parasit Vectors* 8:1–14
- Rioux JA, Carron S, Dereure J, Perieres J, Zeraia L, Franquet E, Babinot M, Gállego M, Prudhomme J (2013) Ecology of leishmaniasis in the South of France. 22. Reliability and representativeness of 12 *Phlebotomus ariasi*, *P. perniciosus* and *Sergentomyia minuta* (Diptera: Psychodidae) sampling stations in Vallespir (eastern French Pyrenees region). *Parasite* 20:34
- Risueño J, Muñoz C, Pérez-Cutillas P, Goyena E, González M, Ortuño M, Bernal LJ, Ortiz J, Alten B, Berriatua E (2017) Understanding *Phlebotomus perniciosus* abundance in south-east Spain: assessing the role of environmental and anthropic factors. *Parasit Vectors* 10: 189
- Romanian National Institute of Statistics (2013) Final results of population census from 2011. Table 8, Ethnic stable population - counties, municipalities, towns, communes. http://www.recensamantromania.ro/wp-content/uploads/2013/07/sR_Tab_8.xls. Accessed 5 September 2018 (in Romanian)
- Rossi E, Bongiorno G, Ciolli E, Di Muccio T, Scalone A, Gramiccia M, Gradoni L, Maroli M (2008) Seasonal phenology, host-blood feeding preferences and natural *Leishmania* infection of *Phlebotomus perniciosus* (Diptera, Psychodidae) in a high-endemic focus of canine leishmaniasis in Rome province, Italy. *Acta Trop* 105:158–165
- Sawalha SS, Ramlawi A, Sansur RM, Salem IM, Amr ZS (2017) Diversity, ecology, and seasonality of sand flies (Diptera: Psychodidae) of the Jenin District (Palestinian territories). *J Vector Ecol* 42:120–129
- Simsek FM, Alten B, Caglar SS, Ozbel Y, Aytekin AM, Kaynas S, Belen A, Kasap OE, Yaman M, Rastgeldi S (2007) Distribution and altitudinal structuring of phlebotomine sand flies (Diptera: Psychodidae) in southern Anatolia, Turkey: their relation to human cutaneous leishmaniasis. *J Vector Ecol* 32:269–279
- Tanase OI, Daraban C, Velescu E, Boghean D, Bocaneti-Daraban F (2018) Symptomatic Leishmaniasis in an Italian Segugio dog from northeastern Romania: a case report. *Iran J Parasitol* 13:673–678
- Tánczos B, Balogh N, Király L, Biksi I, Szeredi L, Gyurkovsky M, Scalone A, Fiorentino E, Grammiccia M, Farkas R (2012) First record of autochthonous canine leishmaniasis in Hungary. *Vector Borne Zoonotic Dis* 12:588–594
- Tarallo VD, Dantas-Torres F, Lia RP, Otranto D (2010) Phlebotomine sand fly population dynamics in a leishmaniasis endemic peri-urban area in southern Italy. *Acta Trop* 116:227–234
- Volf P, Ozbel Y, Akkafa F, Svobodová M, Votýpka J, Chang KP (2002) Sand flies (Diptera: Phlebotominae) in Sanliurfa, Turkey: relationship of *Phlebotomus sergenti* with the epidemic of anthroponotic cutaneous leishmaniasis. *J Med Entomol* 39:12–15

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