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Phlebotomine sand fly population dynamics in a leishmaniasis endemic peri-urban area in southern Italy

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ABSTRACT

A 2-year survey was carried out from May to November 2008 and 2009 to study the sand fly species composition, its seasonal phenology and density in Apulia region (southern, Italy). The study was conducted in a dog shelter located in a new residential urban district where *Leishmania infantum* is endemic. Sand flies were collected using sticky traps from May to November, at about 7-day intervals. Temperature and relative humidity were recorded daily. In December 2008, general environmental improvements (e.g., the ground was covered with gravel and the vegetation present inside the cages was removed to facilitate cleaning) were made in the study area. The most diffused species during the whole study period were *Phlebotomus perniciosus* (2008, $n = 248$, 49.4%; 2009, $n = 254$, 50.6%) followed by *Phlebotomus neglectus* (2008, $n = 76$, 39.8%; 2009, $n = 115$, 60.2%) and *Phlebotomus papatasi* (2008, $n = 5$, 50.0%; 2009, $n = 5$, 50.0%). Four specimens of *Phlebotomus perfiliewi* were collected only in the first year. The number of *Sergentomyia minuta* specimens collected increased considerably in the second ($n = 548$, 86.2%) in comparison to the first year ($n = 88$, 13.8%). The highest number of phlebotomine sand flies was collected in July and August when a mean temperature from 27.09 to 28.02 °C and mean relative humidity from 47.28 to 56.36% rates were recorded. The variations in phlebotomine sand fly species diversity and abundance recorded in this study were related to climatic and environmental factors. Data here presented confirm that sand flies easily adapt to the urban environment and that the may represent a public health concern for *L. infantum* and other pathogen transmission also in similar urban environment of southern Europe.

1. Introduction

Phlebotomine sand flies (Diptera: Psychodidae) are vectors of several zoonotic pathogens causing bartonellosis, leishmaniasis and arboviral diseases (Tesh and Guzman, 1996). In the Old World, about 40 species of *Phlebotomus* have been regarded as proven or suspected vectors of *Leishmania* spp. (Alexander and Maroli, 2003). Canine leishmaniasis due to *Leishmania infantum* is transmitted by different species of *Phlebotomus* and it is regarded as one of the most important canine protozoal diseases of zoonotic concern in the Mediterranean area (Baneth et al., 2008). In Italy, stable endemic foci of canine leishmaniasis by *L. infantum* have been reported from central and southern regions, including Sicily and Sardinia islands (Otranto and Dantas-Torres, 2010). However, in recent years *L. infantum* and its vectors have spread from traditional endemic areas of southern and insular regions to central and northern parts of the country (Maroli et al., 2008; Otranto et al., 2009a).

The Italian phlebotomine sand fly fauna is represented by eight species which include *Phlebotomus perniciosus* Newstead, 1911, *Phlebotomus perfiliewi* Parrot, 1930, *Phlebotomus ariasi* Tonnoir, 1921, *Phlebotomus neglectus* Tonnoir, 1921, *Phlebotomus papatasi* (Scopoli, 1786), *Phlebotomus mascittii* Grassi, 1908, *Phlebotomus sergenti* Parrot, 1917 and *Sergentomyia minuta* (Rondani, 1843) (Maroli et al., 1994a; D'Urso et al., 2004). Of these, *P. perniciosus*, *P. perfiliewi*, *P. ariasi*, and *P. neglectus* have been regarded as proven or suspected vectors of *L. infantum* in Italy, being the first one the major vector of the protozoon (Bettini et al., 1986; Léger et al., 1988; Maroli et al., 1994b; Bongiorno et al., 2003; Ready, 2010). The introduction of *L. infantum* in a previous non-endemic area might be a consequence of increased movement of dogs (e.g., importation of infected dogs) and of environmental changes, which could increase the number of microhabitats potentially suitable to phlebotomine sand flies (Otranto et al., 2009a). A constant monitoring of sand fly vectors in new foci of leishmaniasis is crucial for assessing the geographical expansion of the disease. On the other hand, surveys of sand fly population in traditional leishmaniasis-endemic areas are also required mainly where there is a high prevalence of asymptomatic infection in dogs (Otranto et al., 2009b), which might represent a major public health threat. Although some entomological data on sand flies occurring in southern Italy are available (Puccini et

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Fig. 1. Study area located in a new residential district in Putignano, about 200 m far from the nearest houses (A). During the first year the ground of the cages were covered with grass and bushes. Resting places were built with wood (B). During the second year the ground was covered with gravel and the natural vegetation removed and burned (C).

al., 1977; Maroli et al., 1987, 1994a; Otranto et al., 2007), there is no information on the relationship between environmental and climatic (e.g., temperature and relative humidity) conditions, and seasonality of different sand flies in this region. Hence, the aim of this study was to assess the sand fly species diversity and seasonality in a peri-urban area of southern Italy where *L. infantum* infection in dogs is endemic (Otranto et al., 2010). In particular, the relationship between climate data and the sand fly population has been assessed throughout two consecutive seasons.

2. Materials and methods

2.1. Study area and environment

An entomological survey was conducted in a dog shelter hold by an animal welfare organization in the municipality of Putignano (40°51′N, 17°7′E; 372 m above sea level), Apulia region, southern Italy, where *L. infantum* infection in dogs is high prevalent (Otranto et al., 2010). This municipality has a population of 27,913 inhabitants and it is located in an area with a typical Mediterranean climate (i.e., hot and dry summer and mild wet winter), annual rainfall ranging from 638 to 810 mm, monthly mean relative humidity from 59 to 79%, and mean monthly temperature from 2 to 30 °C. The hottest months (July–August) are characterized by low rainfall (28–42 mm) and daily mean temperature ranging from 16 to 29.5 °C.

The dog shelter chosen for this survey is located in a 10 years old residential district in Putignano, about 200 m far from the nearest houses (Fig. 1A). The study area is connected to the centre of the town by two main streets and many private houses with gardens were present in the neighborhood. The kennel is surrounded by a green area with Mediterranean vegetation and fields with olive and cherry trees and typically delimited by stone walls.

About 100 dogs were present in the shelter and housed in 12 wire mesh cages (approximately 10 m × 20 m) of which 7 had one of the sides delimited by a limestone wall. Each cage contained from three to eight dogs of different sex, age and breed. During the first sampling summer season, the ground of the cages was covered with grass and bushes and resting places built with wood, metal, and plastic pieces were present in each cage (Fig. 1B). Although about half of the dogs present in the shelter were treated with imidacloprid 10% and permethrin 50% spot-on against ectoparasites during the first year only, the environment was not treated with any insecticide throughout the whole study period. In Decem-

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Table 1
Phlebotomine sand flies collected in 2008 and 2009, according to species and sex.

Species	Male		Female		Total	
	2008 No. (%)	2009 No. (%)	2008 No. (%)	2009 No. (%)	2008 No. (%)	2009 No. (%)
Phlebotomus perniciosus	169 (68.1)	222 (87.4)	79 (31.9)	32 (12.6)	248 (49.4)	254 (50.6)
Phlebotomus neglectus	76 (100.0)	112 (97.4)	–	3 (2.6)	76 (39.8)	115 (60.2)
Phlebotomus papatasi	5 (100.0)	3 (60.0)	–	2 (40.0)	5 (50.0)	5 (50.0)
Phlebotomus perfiliewi	2 (50.0)	–	2 (50.0)	–	4 (100.0)	–
Phlebotomus spp.[a]	4 (26.7)	35 (67.3)	11 (73.3)	17 (32.7)	15 (22.4)	52 (77.7)
Sergentomyia minuta	50 (56.8)	292 (53.3)	38 (44.2)	256 (46.7)	88 (13.8)	548 (86.2)
Total	306 (70.2)	664 (68.2)	130 (29.8)	310 (31.8)	436 (30.9)	974 (69.1)

[a] Damaged specimens, unsuitable for morphological identification at species level.

ber 2008, general environmental improvements were made in the study area (Fig. 1C). In particular, the ground was covered with gravel and the old dog resting places were replaced with plastic drums, which served as plastic dog kennels. Finally, the natural vegetation, which was abundant around and inside the cages, was removed and burned to facilitate cleaning.

2.2. Sand fly collection and identification

From May to November of 2008 and 2009, phlebotomine sand flies were collected in the aforementioned dog shelter using sticky traps. Collections were carried out twice a month until the appearance of the first sand fly and then every 7 ± 3 days until the disappearance of the last one. A final collection was conducted 2 weeks later to confirm the absence of sand flies. Collections were performed by using approximately 8 m^2 of white sheets (128 sheets measuring 21 cm × 30 cm) soaked in castor oil (Castor Oil, Carlo Erba spa, Rodano, Milano, Italy). Throughout the study 4736 sticky traps, placed in groups of 16 white sheets, were used. Each group was spaced from each other by a distance between 5 and 15 m. Sticky traps were numbered, attached to the inner surface of the wire mesh cages at about 170 cm from the ground, fixed before sunset (around 6:00 pm) and left *in loco* for two consecutive nights, being recovered in the morning (around 7:00 am) of the third day.

Sand flies caught by sticky traps were firstly immersed in 90% ethanol (to remove oil excess) and then transferred to glass tubes containing 70% ethanol (Maroli and Fausto, 1986). Each sand fly was examined using a stereomicroscope (LEICA MS5, Germany) and separated by sex. For species identification, the external genitalia of males and head and posterior end of abdomen (last two tergites) of females were dissected using entomological needles and cleared with 10% potassium hydroxide solution at room temperature for 2 h. Then, the material was washed with water for 1–2 min, immersed in 10% aqueous solution of glacial acetic acid for 30 min, washed with water for 30 min and finally slide-mounted in Hoyer's solution as recommended by Lewis (1973). Identification was performed using the morphological keys provided by Killick-Kendrick et al. (1991) and Romi et al. (1994).

2.3. Meteorological data

Temperature (°C) and relative humidity (%) were recorded daily by a data logger (HD 206 Delta OHM, Padova, Italy), which was left in the dog shelter during the whole study period. These climate data were recorded every 30 min for a total of 48 records per day. Then, the daily and monthly mean temperature and relative humidity were calculated (Tables 3 and 4).

2.4. Data analysis

The density of phlebotomine sand flies collected by sticky traps was calculated using the formula: Density = number of specimens/m^2 of sticky traps. The sex ratio (male:female) was also calculated for each species collected during this study. Data were compared using Student's *t*-test, considering $P < 0.05$ as significant. Non-parametric data were transformed to logarithmic values before analysis. Pearson (*r*) and Spearman (*rs*) correlations were used to assess the relationship between the number of sand flies and climate variables (mean temperature and mean relative humidity). Correlations between parametric and non-parametric data were performed using Pearson and Spearman, respectively. The normality of the data was assessed using the Lilliefors test. Statistical analyses were performed using the BioEstat 5.0 software (Ayres et al., 2005).

3. Results

On the whole, 1410 phlebotomine sand flies were collected. Five species were identified: *P. perniciosus*, *P. neglectus*, *P. perfiliewi*, *P. papatasi* and *S. minuta* (Table 1). The most abundant species was *S. minuta* ($n = 636$), followed by *P. perniciosus* ($n = 502$) and *P. neglectus* ($n = 191$). In 2008 and 2009, the majority of the phlebotomine sand flies were collected in July and August (Table 2), when the highest monthly mean temperature (i.e., from 27.09 to 28.02 °C) and lowest monthly mean relative humidity rates (i.e., 47.28% to 56.36%) were recorded (Figs. 2 and 3). *P. perfiliewi* and *P. papatasi* were collected in low numbers in 2008, being the former species absent in 2009. The seasonal patterns of phlebotomine sand flies (including all species),

Table 2
Density (specimens/m^2) of phlebotomine sand flies collected in 2008 and 2009, according to genera and sex.

Month	*Phlebotomus*		*Sergentomyia*		Males		Females		Total	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
May	–	<0.1	–	0.4	–	<0.1	–	–	–	0.4
June	1.8	1.8	0.5	1.8	1.5	1.5	0.3	0.3	2.3	3.5
July	6.0	6.1	2.3	9.3	4.2	5.4	1.8	0.7	8.3	15.3
August	14.0	18.5	1.0	22.3	8.9	16.4	5.1	2.1	15.0	40.8
September	2.7	1.9	0.9	1.1	2.4	1.6	0.3	0.3	3.6	3.1
October	0.5	1.5	0.2	0.9	0.3	1.1	0.2	0.4	0.6	2.4
November	0.1	–	–	–	0.1	–	–	–	0.1	–

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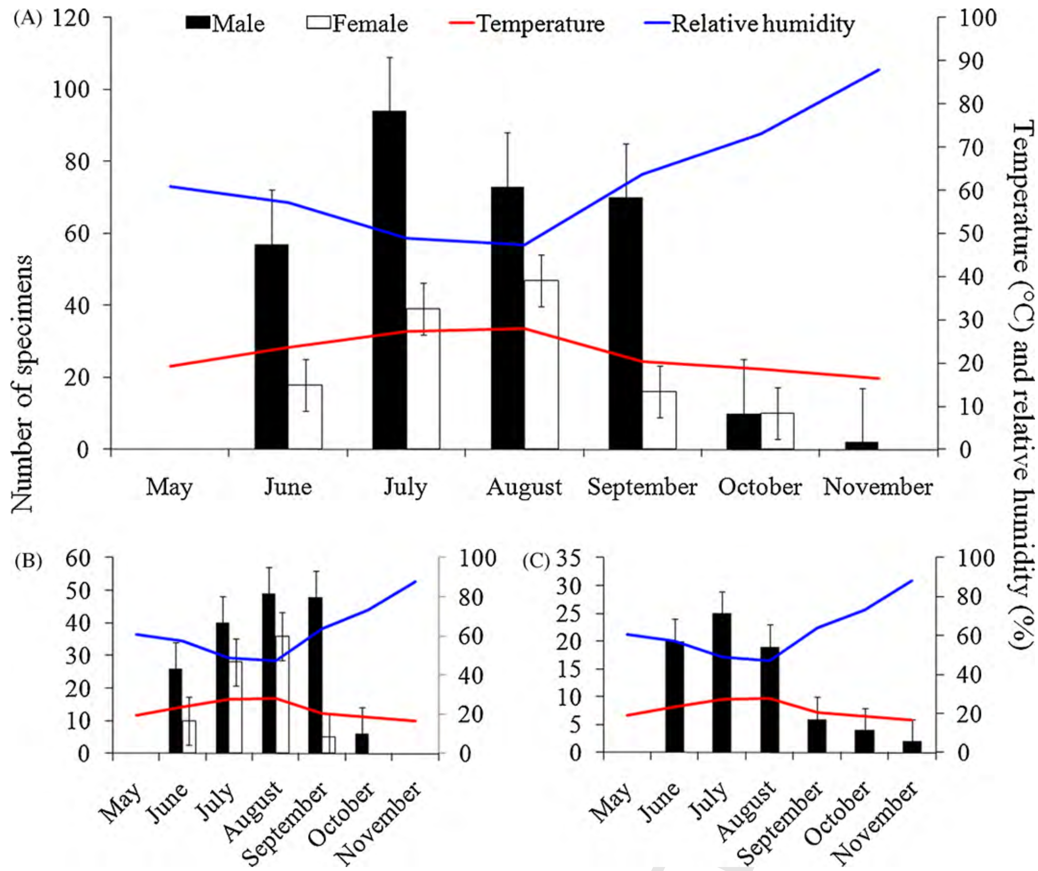

Fig. 2. Total number of sand flies (A), *Phlebotomus perniciosus* (B), and *Phlebotomus neglectus* (C) collected during the first year (2008) and variations in monthly temperature and relative humidity.

P. perniciosus, *P. neglectus* and *S. minuta*, recorded during the 2 years of collections are shown in Fig. 4.

In 2008, the phlebotomine sand flies appeared in the first week of June (Table 3) when the temperature increased and the relative humidity decreased (Fig. 2A). Most of the phlebotomine sand fly species occurred in higher numbers in July whereas *P. perniciosus* was more abundant in August, when the highest monthly mean temperature (28 °C) and the lowest monthly mean relative humidity (47%) were recorded (Fig. 2B). *Phlebotomus* species prevailed in number over *S. minuta*. Indeed, the mean density of *Phlebotomus* species (3.6/m^2) was over fivefold higher than that of *S. minuta* (0.7/m^2). From September onward, when the monthly mean temperature decreased and the monthly mean relative humidity increased after several days of rain (data not shown), phlebotomine sand flies decreased in number, disappearing in the second week of November (autumn).

In 2009, the phlebotomine sand flies were firstly collected in the second half of May (Table 4), remaining present until the second week of October (Fig. 3A). As in 2008, most of the phlebotomine sand flies were collected in July and August, when the highest monthly mean temperature and the lowest monthly mean relative humidity were recorded (Fig. 3A). The days of capture were 52 and 40 in 2008 and in 2009, respectively. Although the capture period was slightly shorter than in 2008, the number of sand flies collected more than doubled. With the exceptions of *P. perfiliewi* (absent in 2009) and *P. papatasi* and *P. perniciosus*, whose number has remained constant, the number of specimens collected in the second year increased for all species, especially for *S. minuta*. In contrast to what occurred in 2008, *S. minuta* was more abundant than all species of *Phlebotomus* species. Again, the mean density of *S. minuta* (5.1/m^2) was higher than that of *Phlebotomus* species (4.3/m^2).

The overall number of phlebotomine sand flies collected in 2008 was lower than in 2009. However, the number of insects collected monthly in both years was not significantly different (Student's *t*-test, $P = 0.62$). In the same way, no difference was found in relation to the number of males and females collected in 2008 and 2009. In relation to meteorological data, the monthly mean tempera-

Table 3
Density, number and sex ratio of phlebotomine sand flies collected in 2008 and the mean daily temperature and relative humidity recorded at each day of collection.

Collection day	Density (specimen/m^2)	No. specimens	M	F	Sex ratio (M:F)	T (°C)	RH (%)
16-May	–	–	–	–	–	18.9	82.8
23-May	–	–	–	–	–	20.0	65.3
30-May	0.1	1	1	–	–	23.9	72.1
06-Jun	0.5	4	2	2	1.0	18.5	89.2
13-Jun	1.3	10	7	3	2.3	20.8	73.0
23-Jun	7.5	60	13	13	1.0	27.7	48.1
11-Jul	6.6	53	17	17	1.0	28.8	58.6
25-Jul	10.0	80	47	22	2.1	26.5	68.3
16-Aug	15.0	120	73	47	1.6	26.7	73.4
06-Sep	9.5	76	61	15	4.1	31.0	49.4
17-Sep	0.3	2	2	–	–	16.9	75.3
27-Sep	1.0	8	7	1	7.0	14.5	90.9
03-Oct	1.8	14	6	8	0.8	16.8	73.8
10-Oct	0.3	2	–	2	–	18.4	98.4
18-Oct	0.3	2	2	–	–	18.8	97.4
25-Oct	0.3	2	2	–	–	16.7	95.9
31-Oct	0.3	2	2	–	–	19.4	87.3
08-Nov	–	–	–	–	–	16.0	98.8
15-Nov	–	–	–	–	–	13.8	99.3

M, male; F, female; *T*, temperature; RH, relative humidity.

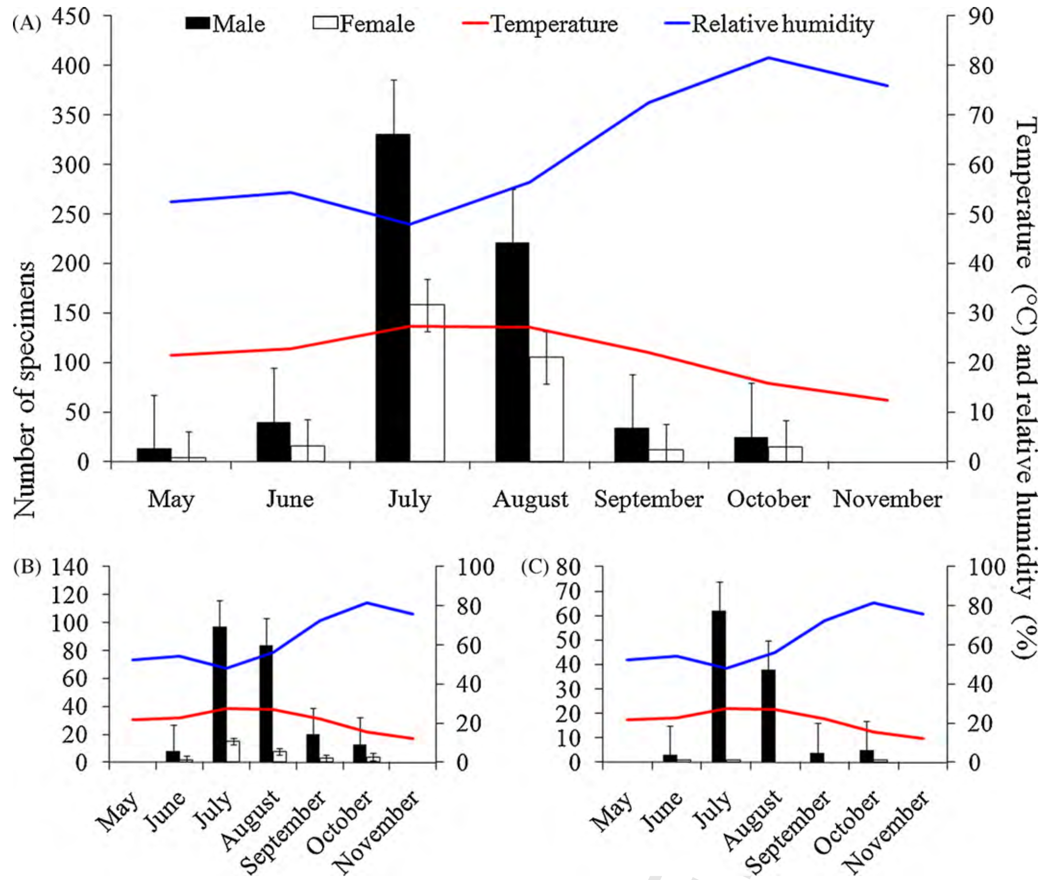

Fig. 3. Total number of sand flies (A), *Phlebotomus perniciosus* (B), and *Phlebotomus neglectus* (C) collected during the second year (2009) and variations in monthly temperature and relative humidity.

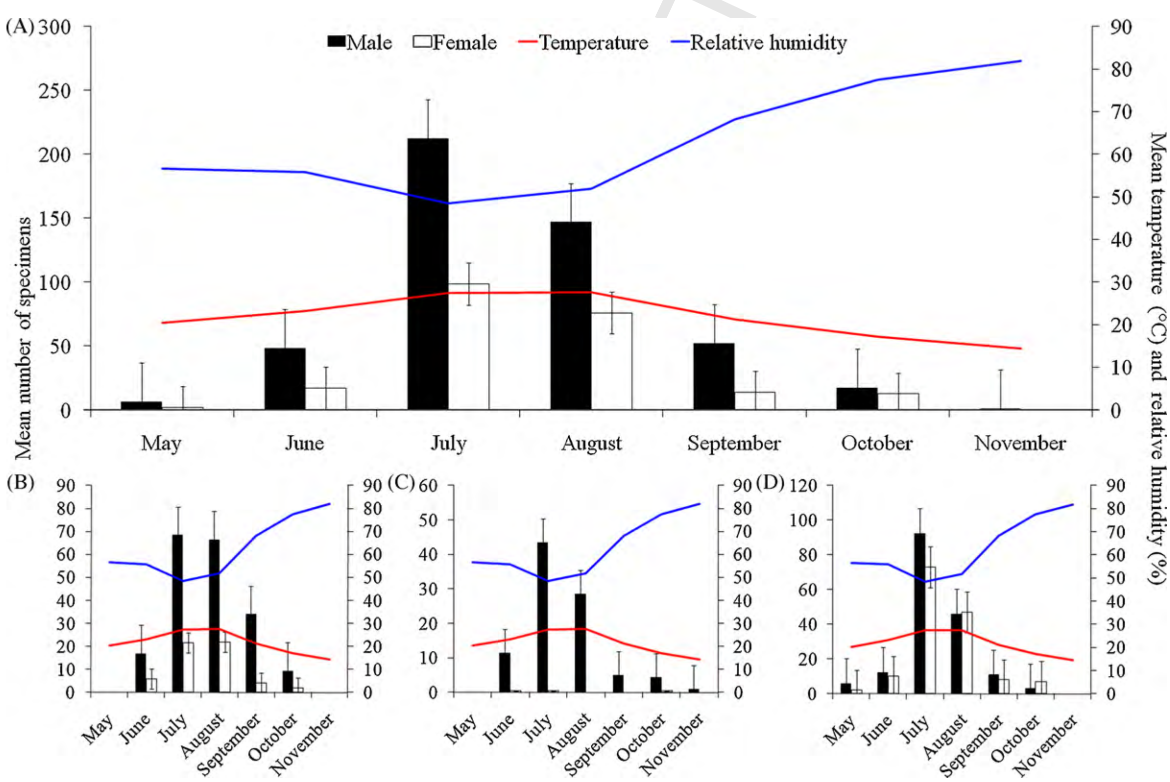

Fig. 4. Mean number of sand flies (A), *Phlebotomus perniciosus* (B), *Phlebotomus neglectus* (C) and *Sergentomyia minuta* (D) collected in 2008 and 2009 and variations in monthly temperature and relative humidity.

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Table 4
Density, number and sex ratio of phlebotomine sand flies collected in 2009 and the mean daily temperature and relative humidity recorded at each day of collection.

Collection day	Density (specimen/m^2)	No. specimens	M	F	Sex ratio (M:F)	T (°C)	RH (%)
10-May	–	–	–	–	–	21.6	51.0
18-May	–	–	–	–	–	22.6	54.2
20-May	0.3	2	2	–	–	26.0	46.9
22-May	0.9	7	3	4	0.8	27.7	45.1
25-May	0.9	7	7	–	–	27.2	42.1
27-May	0.1	1	1	–	–	25.3	47.8
9-Jun	1.6	13	9	4	2.3	26.9	45.7
16-Jun	5.4	43	31	12	2.6	29.4	33.7
30-Jun	7.6	61	49	12	4.1	24.2	65.3
10-Jul	6.3	50	29	21	1.4	24.5	54.6
20-Jul	28.8	230	149	81	1.8	26.5	43.7
29-Jul	18.5	148	104	44	2.4	28.4	48.3
22-Aug	40.8	326	221	105	2.1	28.1	51.9
4-Sep	2.3	18	13	5	2.6	27.4	61.6
18-Sep	4.0	28	21	7	3.0	22.6	78.1
30-Sep	4.0	32	17	15	1.1	21.5	74.5
12-Oct	8.0	8	8	–	–	14.8	77.4
24-Oct	–	–	–	–	–	15.3	96.1
2-Nov	–	–	–	–	–	11.6	72.2

M, male; F, female; *T*, temperature; RH, relative humidity.

ture and monthly mean relative humidity recorded in 2008 did not differ statistically from that of 2009 (Student's *t*-test, $P = 0.84$ for temperature and $P = 0.97$ for relative humidity).

Overall, correlation analysis revealed a strong positive association ($rs = 0.89$, $P < 0.05$) between the monthly number of sand flies collected and monthly mean temperature (Fig. 5A). On the contrary, there was a moderate negative correlation ($rs = -0.82$, $P < 0.05$) between the monthly number of sand flies collected and monthly mean relative humidity (Fig. 5B). When the data was analyzed separately by year and by sand fly genera or species, the number of specimens collected remained positively correlated with temperature and negatively correlated with relative humidity, although these correlations were not always statistically significant (Table 5).

4. Discussion

The occurrence of phlebotomine sand fly species in the urban area of southern Italy indicates the potential risk of *L. infantum* infection to humans and animals living there. Although the five species (i.e., *P. perniciosus*, *P. neglectus*, *P. perfiliewi*, *P. papatasi* and *S. minuta*) collected in this study have been already identified in previous entomological surveys in southern Italy (Puccini et al., 1977; Maroli et al., 1987, 1994a; Otranto et al., 2007), it was unexpected to collect all of them at the same time, in the same collection site. These results indicated a high degree of species richness in the sand fly population occurring in this urban area of southern Europe.

Table 5
Correlation between the number of phlebotomine sand flies collected and monthly mean temperature (*T*) and monthly mean relative humidity (RH) recorded in 2008 and 2009.

Variables	2008		2009	
	Pearson	*P*-value	Spearman	*P*-value
Phlebotomus spp. + *S. minuta* × *T*	0.90	0.01	0.93	0.00
Phlebotomus spp. × *T*	0.92	0.00	0.87	0.01
S. minuta × *T*	0.61	0.15	0.98	0.00
P. perniciosus × *T*	0.89	0.01	0.72	0.07
P. neglectus × *T*	0.92	0.00	0.70	0.08
Phlebotomus spp. + *S. minuta* × RH	−0.80	0.03	−0.54	0.22
Phlebotomus spp. × RH	−0.81	0.03	−0.46	0.29
S. minuta × RH	−0.57	0.18	−0.71	0.07
P. perniciosus × RH	−0.79	0.03	−0.25	0.59
P. neglectus × RH	−0.76	0.05	−0.22	0.64

However, the sand fly population collected in the study site changed over the two collection seasons. Indeed, during the first year of study, *P. perniciosus*, *P. neglectus*, *P. perfiliewi* and *P. papatasi* and a relatively low number of *S. minuta* were found. Conversely, during the second year, *P. perfiliewi* was not collected and a significant increase in the number of *S. minuta* was recorded. As a matter of fact, the high abundance of *P. perniciosus* over the 2 years confirms that this species is well adaptable not only to sylvatic environments but also to the urban and peri-urban areas (Biocca et al., 1977; Maroli et al., 1994a). The notable biological plasticity of *P. perniciosus* explains why it is one of the most ubiquitous vector species in southern Europe (Ready, 2010). This data is of public health relevance considering that *P. perniciosus* is the major vector of *L. infantum* in the Old Word (Ready, 2010).

Following *P. perniciosus*, *P. neglectus* was the most abundantly collected species in both years, with a substantial increase in 2009. This result is in line with previous reports of *P. neglectus* in Italy (Maroli et al., 2008) where this species has been found at the sea level, along the coastal foci of southern Italian regions, and in hilly areas up to 1300 m above sea level (Maroli et al., 2002). This sand fly species has been collected both in sylvatic and domestic or peridomestic environments (Maroli et al., 2002). Differently, *P. papatasi* has been found in low but constant number during the 2 years of study. The range of *P. papatasi* prevalence (i.e., from 0.5 to 1% in 2009 and 2008, respectively) is slightly higher than those previously recorded in Italy (i.e., from 0.05 to 0.4%) throughout 18 years of sand fly sampling (Maroli et al., 1994a). Indeed, this species has adapted to live in specific habitats in urban environment as chicken houses, wineries, houses made of bricks and clay, manure heaps, stables, and latrines (Feliciangeli, 2004). Therefore, this species most likely found in the kennel and in houses in the neighborhood suitable sites for its survival and reproduction. Since a few specimens ($n = 4$) of *P. perfiliewi* were captured in 2008 no conclusion on the phenology of this species can be drawn. Nevertheless, the retrieval of this species in an urban area is not a surprising finding because it occurs in domestic settings and around human dwellings (Maroli et al., 1994a). Interestingly, the high density of *S. minuta* over both years and, again, its significant increase during the second year is an unexpected finding considering that this species is usually less abundant in human settings (Maroli et al., 1994a). Indeed, *S. minuta*, feeds on cold-blooded animals and finds the best conditions for its development in sylvatic environments (Lewis, 1978). Considering that no significant variation in the monthly mean temperature and relative humidity were recorded in 2008 and 2009, it is unlikely that climatic factors affected the diversity and abundance of the sand fly species herein considered. With some exceptions (e.g., absence of *P. perfiliewi* and high abundance of *S. minuta* in 2009), the phlebotomine sand fly population remained somewhat constant. Indeed, the general improvements made in the kennel in December 2008 and the suspension of the treatment (from March 2009 onwards) of part of the dogs with spot-on ectoparasiticides did not affect the phlebotomine sand fly population in the study area. Interestingly, the number of *S. minuta* collected doubled in 2009. Therefore, one could argue that the suspension of the dog treatment and the general improvements made in the kennel could have positively affected the population of *S. minuta*. However, this hypothesis is unlikely because these changes should have also affected other phlebotomine sand fly species. Additionally, based on feeding habits of the genus *Sergentomyia* (Lewis, 1978), the expected impact of the treatment of dogs with ectoparasiticides on *S. minuta* should be minor. Indeed, these data should be interpreted with cautious because other unrecorded climate variables (e.g., wind, precipitation) and environmental changes (e.g., changes in the density of lizards within the kennel's area) could also have influenced the increased number of specimens collected in 2009. Phlebotomine sand flies were collected in the study area

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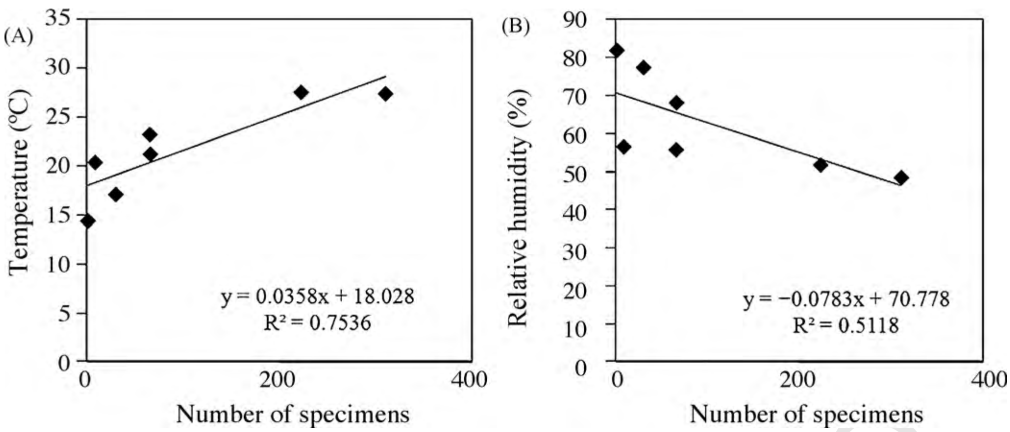

Fig. 5. Positive correlation between the number of sand flies collected monthly and monthly mean temperature (A) and negative correlation between the number of sand flies collected and monthly mean relative humidity (B) in both years of study.

from the second half of May to early October confirming that these insects have a well-defined seasonal activity, peaking during July and August in southern Italy. These data are in accordance with previous findings from central and northern Italy (Bongiorno et al., 2003; Rossi et al., 2007; Maroli et al., 2008). In particular, the most suitable period for sand fly collection is July and August, when the mean temperature is high and the mean relative humidity is low. Indeed, the risk of being bitten by sand flies is higher during summer, when there is also an increased movement of tourists to southern Italy and, in general, to the Mediterranean area. Incidentally, the introduction of leishmaniasis into non-endemic areas of northern Europe (e.g., Germany) has been regarded, in part, as a consequence of tourists returning with their dogs that have been infected in endemic areas in the Mediterranean region (Menn et al., 2010). Our data further indicate that the phlebotomine sand fly distribution and abundance are not affected only by climate factors, but also by other environmental factors (e.g., presence and abundance of potential breeding sites) and availability of suitable hosts, which ultimately impact on their ecology. Accordingly, the spread of leishmaniasis into urban areas is favored by the adaptability of phlebotomine sand flies to human-modified environments. Indeed, the understanding of the interactions occurring between urban environmental changes and phlebotomine sand fly vectors is a prerequisite for the development of appropriate preventive actions and control strategies.

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