Seasonal dynamics of six phlebotomine sand fly species proven vectors of Mediterranean leishmaniasis caused by *Leishmania infantum*

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Background

Phlebotomine sand flies are the unique haematophagous insects proven to transmit leishmaniases. Of approximately 900 species estimated to exist, less than a hundred, belonging to *Phlebotomus* and *Lutzomyia* genera, are proven or suspected vectors of human disease in the Old and New Worlds, respectively. *Leishmania infantum* is the agent of visceral leishmaniasis and sporadic cutaneous leishmaniasis in the Mediterranean subregion including southern Europe, northern Africa and parts of Asia. A dozen of *Phlebotomus* species have been implicated in parasite transmission, of which 8 have been conclusively incriminated as vectors. All these species but *P. balcanicus*, which belongs to the subgenus *Adlerius*, are members of the *Larroussius* subgenus.

Effects of long-term climate changes on the geographical expansion of *L.infantum* vectors towards northern latitudes and/or higher altitudes have been predicted and actually observed in some places, such as northern Italy, French and Spanish Pyrenees, and in Rhineland-Palatinate, Germany. The activity period of Mediterranean adult sand flies is typically seasonal. Because seasonal phenomena are also very sensitive to variations in temperature, phenological observations, besides geographical dispersion parameters, may also provide high resolution of ongoing climate changes. Available information on sand fly seasonal dynamics in the Mediterranean region is patchy, being disperse in time and space. Several investigations have been performed during one season (rarely two) in single sites and in different years. Hence, recent and accurate knowledge of the vector populations dynamics is required as starting baseline for prospective investigations on seasonal climate changes that may have an impact on Mediterranean leishmaniasis transmission. Because investigations of this type are prone to several confounding parameters, prerequisites would be: a) to perform studies in a relatively short period (i.e. a time range of a few years) to minimize the effects of the ongoing temperature increase; b) to replicate studies in the same sites for more than one season to minimize the effects of local climate changes; and c) to carry out studies over a wide geographical area, in order to provide general data from different latitudes and longitudes.

Aims

In the frame of an European Commission-funded project (EDENext), in 2011-2014 a consortium of partners from 8 Mediterranean countries endemic for *L.infantum* have carried out entomological investigations in representative sites using standard sand fly collection methods for at least 2 consecutive years. Here, we report observational data from about 99,000 specimens, resulting in the description of seasonal dynamics of 6 *L.infantum* vector species over a wide geographical range spanning from Portugal at west to Georgia at east, namely: *P.perniciosus* (Portugal, Spain and Italy); *P.ariasi* (France); *P.neglectus* (Greece); *P.tobbi* (Cyprus and Turkey); *P.balcanicus* and *P.kandelakii* (Georgia).

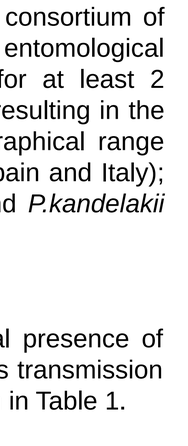
Study areas

In each country, one or more locations were identified on the basis of the historical presence of proven phlebotomine vector(s) and the evidence of human and/or canine leishmaniasis transmission in the area. Geographical coordinates and altitude of study regions and sites are shown in Table 1.

Table 1. Geographical coordinates and altitude of 37 study sites

Country	Region/District/Site	No. of sites	Latitude	Longitude	Elevation (m a.s.l.)
Portugal	Lisbon Metropolitan Region/Setúbal and Lisbon	11	From 38 28'37''N to 38 44'51''N	From 9 16'52''W to 8 45'2''W	3 – 330
	Algarve/Faro	11	From 37 3'27''N to 37 14'20''N	From 8 37'45''W to 7 26'34''W	10 – 74
Spain	Autonomous Community of Madrid/Fuenlabrada	1	40 17'53''N	3 47'31''W	635 – 691
France	Languedoc-Roussillon/ Gard/Roquedur-le-haut	1	43 58'23''N	3 39'26''E	603
Italy	Latium/Rome/Frascati	1	41 50'34''N	12 41'57''E	192
Greece	Crete/Heraklion/Fodele	1	35 22'52''N	24 57'29''E	40 – 70
Cyprus	Paphos/Steni	1	34 59'54'' N	32 28'17'' E	200
Turkey	Aegean/Aydin/Bascayir	1	37 57'35''N	28 04'03''E	427
	Cukurova/Adana	6	From 37 17'59''N to 37 26'01''N	From 35 31'01''E to 35 39'27''E	150 – 280
Georgia	Tbilisi/Gldani-Nadzaladevi and Isani-Samgori	3	From 41 42'01''N to 41 44'08''N	From 44 48'59''E to 44 49'30''E	495 – 603

Trapping methods and identification



A standardized protocol for trapping methods and periodicity of collections was established. Priority was given to CDC miniature light traps, placed in each collection site for a minimum of 2 consecutive nights per month. Sticky traps were also used in several sites, set monthly for at least 2 consecutive days. Temperature and humidity were recorded daily during the trapping period. A variety of domestic and peri-domestic habitats were surveyed in rural, village or town areas by means of indoor and/or outdoor collections. Collected specimens were preserved in ethanol pending species identification, performed morphologically using standard keys.

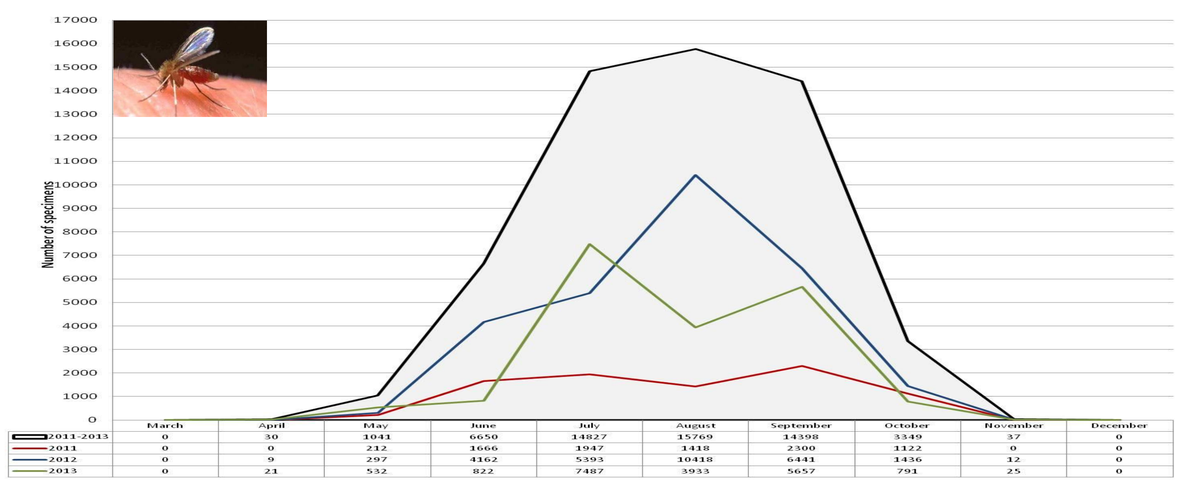
Results

A total of 99,195 sand fly specimens (59.8% males) were trapped, of which 95,384 were correctly identified as belonging to *Phlebotomus* and *Sergentomyia* species. *Phlebotomus* accounted for 18 species of 5 subgenera: 1 to 3 species were collected in western Mediterranean, and 3 to 9 species in eastern Mediterranean sites. As expected per study objectives, higher densities were recorded for proven *L.infantum* vector species in each site. The analysis of seasonal dynamics was performed for 56,101 vector specimens, trapped respectively in 2011 (8,665 specimens from 6 countries: Portugal, France, Italy, Greece, Turkey and Georgia), 2012 (28,168 specimens from all 8 countries) and 2013 (19,268 specimens from 6 countries (Portugal, Spain, France, Greece, Cyprus and Georgia).

An overview of all vectors

Fig. 2 shows the cumulative 2011-2013 abundance trend and the trends corresponding to each year. The former consists of an almost perfect bell-shape curve, having a wide peak center (80%) corresponding to July-September. Most of the curve (99%) is included between May and October, while flat tails (0.5% each) are found in April and November. Trappings performed in March and December were negative for sand flies. This homogeneous curve results from the combination of 3 yearly patterns that look very different each other. In 2011, because of low abundance, no sand flies were trapped in April and November and the pattern did not show any sharp peaks. The 2012 trend was mostly mono-modal with a sharp peak in August; by contrast, the 2013 trend was bi-modal including two sharp peaks in June/July and in September.

Figure 2. Seasonal dynamics overview including all *L.infantum* vector species

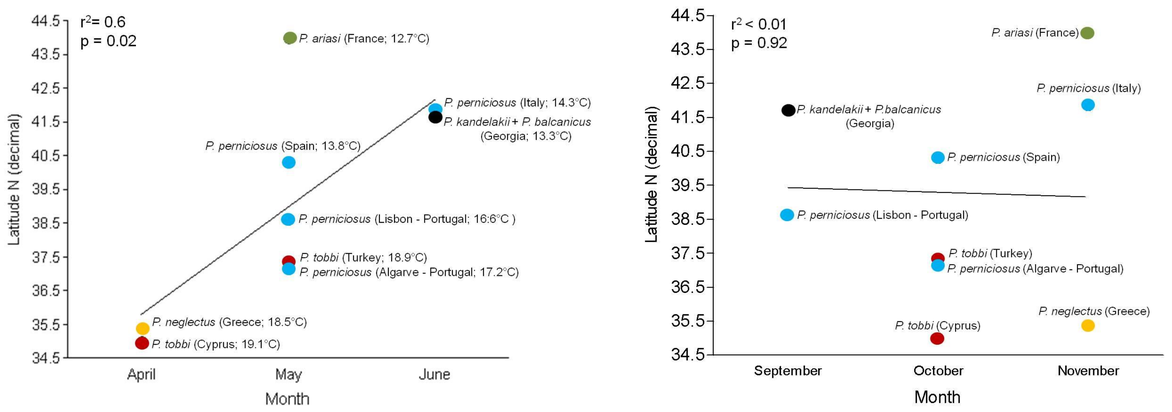


	March	April	May	June	July	August	September	October	November	December
2011-2013	0	30	1041	6650	14827	15769	14398	3349	37	0
2011	0	0	212	1666	1947	1418	2300	1122	0	0
2012	0	9	297	4162	5393	10418	6441	1436	12	0
2013	0	21	532	822	7487	3933	5657	791	25	0

Vector phenology by species and site

The relationship between the period of appearance/disappearance of sand flies and the latitude or average annual temperature of sites, was evaluated using data from cumulative years. When used separately in the analysis, latitude and average annual temperature gave similar results. As shown in Fig. 3 (left), with the exception of *P.ariasi* in France, a significant correlation was found between latitude and the first month of sand fly collection. Some species have appeared earlier at lower latitude, and later at higher latitude. Conversely, adults disappearance was apparently less influenced by latitude (or average annual temperature) since each species per site ended the activity unevenly in a period from September through November (Fig.3, right).

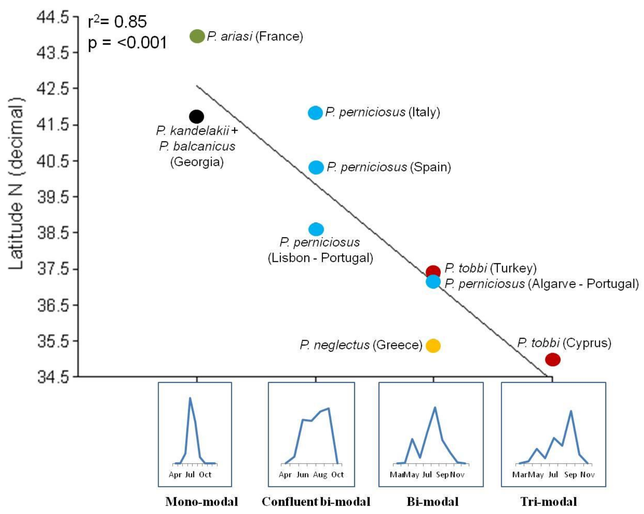
Figure 3. Month of vector appearance (left) and disappearance (right) vs site latitude



Monthly abundance by species and site

Four types of cumulative density trends were identified in each site: 1) A mono-modal trend; 2) A trend resulting from the confluence of 2 density peaks; 3) A bi-modal trend, with a first minor peak; 4) A tri-modal trend, with peaks increasing in magnitude. They were also associated with latitude (Fig.4). Temperatures recorded have been analysed in each site in relation to abundance trends, both within the same season and between different years. We did not detect any meaningful association between temperatures registered at the beginning of the sand fly activity and the occurrence and magnitude of peaks, nor between average temperatures registered in various periods from June to September of different years, and the respective fly abundances.

Figure 4. Type of abundance trend vs latitude



Conclusions

Sand fly abundance and seasonal trends varied greatly between years in the same sites, confirming that local climate changes may affect reliable and meaningful conclusions on phlebotomine dynamics. More realistically, we found that cumulative collections performed in different years from the same site can provide potential and homogeneous patterns of vectors behavior. We found that potential risk for *L.infantum* transmission was basically from June through October in the Mediterranean region, and that such a risk was associated with density waves of adults which are more frequent in southern territories.

This work was supported by EDENext, a collaborative project of the 7th FP (2011-2014) funded by the European Commission under the DG Health; Contract Number: 261504