Distribution and altitudinal structuring of phlebotomine sand flies (Diptera: Psychodidae) in southern Anatolia, Turkey: their relation to human cutaneous leishmaniasis

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ABSTRACT: The two Old World genera, *Phlebotomus* and *Sergentomyia,* were both recorded in southern Anatolia in Turkey. *Phlebotomus* species predominated and comprised about 93% of the entire collection (3,172 specimens). Out of the sixteen species identified, two belonged to the genus *Sergentomyia*: *S. dentata* and *S. theodori*. The remaining fourteen species in the genus *Phlebotomus* were grouped under four subgenera including some species that are elsewhere known to act as vectors of human cutaneous leishmaniasis. Most of the *Phlebotomus* were *P. tobbi* (32.5%), but *P. papatasi*, *P. transcaucasicus, P. halepensis, P. galilaeus, P. sergenti, P. syriacus, P. neglectus, P. simici, P. alexandri, P. similis, P. jacusieli, P. perfiliewi,* and *P. brevis* were also identified. There were two associations of sand fly fauna with altitudinal gradient; the first one at relatively higher altitudes and the second one at lower altitudes. The transition between these two assemblages was within the range of 800-1,000 m. It is likely that Adana and Hatay provinces are transitional areas between western and eastern Anatolia. Mountains do not appear to be important geographical barriers for sand fly distribution. We also found that the proven vector *P. sergenti* is a widely distributed species throughout southern Anatolia and this species, together with its closely related species *P. similis,* shows sympatry in Konya Province. ***Journal of Vector Ecology* 32 (2): 269-279. 2007.**

Keyword Index: Sand flies, geographical distribution, altitudinal structuring, cutaneous leishmaniasis, southern Anatolia, Turkey.

INTRODUCTION

Phlebotomine sand flies are vectors of pathogenic organisms such as *Leishmania* in Anatolia, Turkey, which has been a major endemic focus of leishmaniasis for many years. The area in which they occur is an important interface between the Palaearctic and Afrotropical zoogeographic regions. Located between the Taurus, Amanos, and Anti-Taurus mountains, the southern part of Anatolia represents a geographical crossroad for sand fly dispersion between western and eastern Anatolia, showing various ecological, geographical, and climatic differences which are important in the epidemiology of leishmaniasis.

Two clinical types of leishmaniasis exist in Turkey. Human cutaneous leishmaniasis (HCL) caused by *Leishmania tropica* and *L. infantum* (Serin et al. 2005), is highly endemic in south and southeast Anatolia. Human visceral leishmaniasis (VL), caused by *L. infantum,* is endemic along the Aegean and Mediterranean coasts and occurs sporadically in other regions (Ozbel et al. 1995, Ok et al. 2002, Volf et al. 2002, Yaman and Ozbel 2004). According to the Turkish Ministry of Health, within a period of five years (2000-2004), 127 cases of human visceral leishmaniasis (HVL) and 11,547 cases of cutaneous leishmaniasis were reported in Turkey, with 57% and 97% of these occurring in the southern Anatolia and Mediterranean Regions, respectively.

Previous studies of sand fly fauna in Turkey identified 19 *Phlebotomus* species (or subspecies recently raised to species level) belonging to the *Phlebotomus* Rondani, 1840, *Adlerius* Nitzulescu, 1931, *Larroussius* Nitzulescu, 1931, and *Paraphlebotomus* Theodor, 1948 subgenera. There are five *Sergentomyia* species belonging to the subgenus *Sergentomyia* Franca and Parrot, 1920 (Alptekin et al. 1999, Volf et al. 2002, Alten et al. 2003, Yaman and Ozbel 2004, Toprak and Ozer 2005). Nine of these species are proven or probable vectors of the parasites causing human leishmaniasis in the Old World (Killick-Kendrick 1990).

Ecological studies of sand flies in Turkey show that the altitude and bioclimatic structure have an important impact on the distribution of sand fly species (Belen et al. 2004, Erisoz and Alten, 2005, Belen and Alten 2006). The altitude itself is not an ecological factor, but it can act on sand fly distribution through the diversity of habitats, relief, and through the gradient on climate that it offers. Guernaoui et al. (2006) showed that altitude is one of the most important factors on distribution and structuring of the sand fly species.

Determining the faunistic composition and distribution patterns of sand flies can aid in the incrimination of

vector species, and the distribution of sand fly vectors of leishmaniasis can indicate whether the disease in a particular area is enzootic, zoonotic, or anthroponotic. The current study mainly reports the sand fly fauna, species composition, and distribution in southern Anatolia, Turkey, with the aim of investigating and quantifying the possible effects of altitude and physical barriers on the sand fly populations in relation to the distribution of leishmaniasis. Allopatric/sympatric speciation of the two closely related species, *Phlebotomus sergenti* Parrot, 1917 and *Phlebotomus similis* Perfiliew, 1963, is also discussed.

MATERIALS AND METHODS

Study areas

Field studies were performed in an area of approximately 140,000 km^2, containing plains, rivers, streams, and high mountains, in southern Anatolia from April to October of 2004 and 2005, the period when adult sand flies were active in the study areas. The land that forms the study area is bordered by the Mediterranean Sea to the south, West Taurus Mountains to the west, the mountain ranges of Taurus and Anti-Taurus to the north, and the Amanos Mountains to the east. There are three important mountain ranges that can serve as geographical barriers for species distribution, and four main gaps were recognized among these barriers (Figure 1). The study area was separated into five sub-regions including nine provinces according to these barriers: Konya, Nigde, Kayseri, Adana, Osmaniye, Hatay, Kahramanmaras, Malatya, and Adiyaman (Table 1). Because they had a single sampling site in each, the last two provinces were excluded from the analyses.

Sand fly collections

In each of the seven study areas, sand flies were collected using CDC light traps, sticky papers, and mouth aspirators. Sampling was conducted at 90 sites and 360 sampling points, distributed around the circumferences of the provinces, with an altitude ranging between 0-1,600 m above sea level. In each sampling site, a total of two light traps were set up inside and outside of houses. They were operated between 18:00 and 07:00 once a month during the collection period. Sticky traps in each sampling site prepared from parchment paper (20 x 20 cm) coated with castor oil were placed in various biotopes; inside and around human dwelling and animal housing, close to the vegetation and crevices in walls. In restricted biotopes, such as ventilation shafts of termitaria, rock crevices, rodent burrows, and caves, the traps were rolled into cylinders before they were inserted into the cavities. Fifteen traps were set up in each sampling site at 18:00 and collected after 13-15 h. Mouth aspirators were used for daytime collection of sand flies resting in the houses, barns, and caves. The walls and ceilings of bedrooms, closets, kitchens, and toilet enclosures in each house were inspected.

The specimens collected by light traps and aspirators were directly transferred to 96% ethanol in the field and labeled accordingly. Specimens caught by sticky paper traps were immersed first in 96% ethanol to remove the oil, transferred to 90% ethanol, cleared in lactophenol, and mounted. All specimens were placed in Berlese medium on labeled slides for identification. Before mounting, the head and genitalia of the sand flies were cut, the body parts removed from each specimen with forceps, and the wings stained for easier viewing of veins using Belen's Method (Belen et al. 2004) for further morphometric analysis. The thoraces were ground up and homogenates were stored

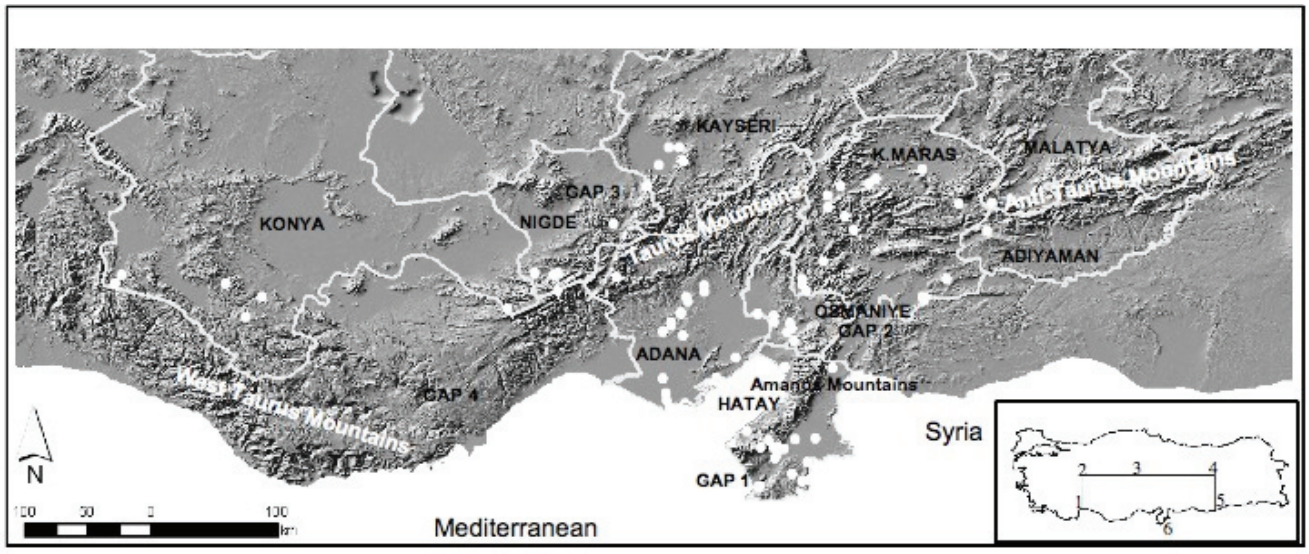

Figure 1. Map of the study area (138676 km^2) showing the main sites sampled, the main geographical barriers, and gaps between the sites [Coordinates: Latitude (DMS)-Longitude (DMS); 1: 36°43'N-32°28'E, 2: 28°22'-31°16'E, 3: 38°22'N-34°39'E, 4: 37°51'N-37°39'E, 5: 37°24'N-38°04'E, 6: 35°47'N-36°07'E]. White circles indicate the sampling localities.

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P.sergenti   cgcagctaac tgtgtgaaat cgtgtgaact gcaggacaca tgaacatcga  50
P.similis    cgcagctaac tgtgtgaaat cgtgtgaact gcaggacaca tgaacatcga  50

P.sergenti   cattttgaac gcatattgcg gtccatgcaa aagtttaaac ttgtttaaac 100
P.similis    cattttgaac gcatattgcg gtccatgcaa aagtttaaac ttgtttaaac 100

P.sergenti   tgcatggacc acgtatggtt gagtatcgta aatattaagc aattgaaatt 150
P.similis    tgcatggacc acgtatggtt gagtatcgta aatattaagc aattgaaatt 150

P.sergenti   gttttttttc ttctatttct ctata***** ***gaaaaag aaaacattgg 192
P.similis    gttttttttt tctttctatt ttttataga aaaaaaaaag aaaacattgg 200

P.sergenti   agttatgaaa t**tttttt catgctctta ata**tgtat taaagtatat 238
P.similis    agttatgaaa ttttttttt catgctctta atacatatt* *aaagcatat 248

P.sergenti   ttgaatgtac ccaatatata tatatattaa attaaaaaga atatatgtgg 288
P.similis    ttgaatgtac ccaatatata taaatattaa attaaaaaga atatatgtgg 298

P.sergenti   tatatcatag tcattgaatt atatcttgcc attgttatac agaacgtata 338
P.similis    tatatcatag tcattgaatt aaatcttgac attgttatac aaaacgtata 348

P.sergenti   tatattttat atttaatata gggattattc atataagaaa atgtgcaaaa 388
P.similis    tatactttat tttaatata gggagtattc atataagaaa atgtgcaaaa 398

P.sergenti   tagaaaa*** ttattttaat tgcgatctca actcatacgt gactaccccc 435
P.similis    tagcaaaaaa ctattttaat tgcgatctca actcatacgt gactaccccc 448

P.sergenti   tgaatttaag catattttta agcggaggaa aagaaactaa ccagg       480
P.similis    tgaatttaag catattttta agcggaggaa aagaaactaa ccagg       493
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Figure 2. ITS2 sequences alignment with flanking 5.8S and 28S rDNA. Base differences are denoted by bold letters. Gaps are denoted by asterisks.

at -80° C for further molecular studies. Identification was based on the morphology of male and female genitalia using the identification keys of Theodor (1958), Artemiev (1980), Lewis (1982) and Killick-Kendrick et al. (1991). At the end of the field study, a total of 3,172 adults was used in the subsequent analysis.

Molecular studies

Comparison and identification of *P. sergenti* and *P. similis* specimens collected from Konya Province, Hatay Province, and Cukurova plain using molecular techniques followed the methods of Depaquit et al. (2000) and are described briefly below.

Extraction of genomic DNA from individual sand flies was performed by using the modified phenol-chloroform method. Individual thoraces were first immersed in liquid nitrogen for 5 min and crushed in 250 µl lysis buffer (25 mM NaCl, 5mM EDTA, 25 mM Tris-HCl pH 8). The sand fly homogenates were incubated with 5 µl proteinase K at 60° C for 16-18 h. DNA was extracted with equal volumes of buffered phenol, Phenol-chloroform-isoamyl alcohol (this mix contains half phenol and half chloroform isoamyl 23:1), and finally chloroform-isoamyl alcohol (23:1). DNA was precipitated with 1/10 volume 3M sodium acetate and 1 volume isopropanol and stored at -20° C for 16-18 h. After centrifugation, the pellet was washed, dried, and resuspended in 20-30 µl double distilled, sterile water.

Polymerase chain reactions (PCR) were performed in 50 µl reaction volumes containing 10 µl genomic DNA, 1x PCR buffer (75 mM Tris- HCl pH 8.8 at 25° C, 20 mM ammonium sulfate $(NH_4)_2SO_4$ and 0.01% Tween-20), 4 mM magnesium chloride, 0.2 mM deoxynucleotide mixture (dTTP, dATP, dCTP, dGTP), 1.25 units *Taq* DNA polymerase and 2 µM of each primer. Primer sequences are C1a: 5' –CCTGGTTAGTTTCTTTTCCTCCGCT-3' and JTS3: 5' –CGCAGCTAACTGTGTGAAATC -3'. Temperature cycling was performed using Thermal Cycler (Thermo Hybaid; Authorized Thermal Cycler for PCR, UK). Thermal cycling included denaturation at 94° C for 2 min, followed by 35 cycles of denaturation at 94° C for 30 s, annealing at 57° C for 30 s. An elongation at 72° C for 2 min completed DNA amplification. PCR was checked by electrophoresis in 2% agarose gel containing ethidium bromide.

PCR products obtained from individual sand flies were sequenced using the AB version kit (Applied Biosystems PRISM Big Dye Terminator v 0.1 Ready Reaction Cycle Sequencing Kit). Differences between the species were compared visually in which defined sequences for *P. sergenti* and *P. similis* were used according to the method of Depaquit et al. (2000).

Data analysis

Ecological comparisons between the sub-regions in the study area and between different altitudes were performed with the computer program: PAST, Palaentological Statistics, ver. 1.25 (Hammer et al. 2006). Shannon-Wiener species diversity index was used to calculate species diversity. The formulae and their rationale in the present study are summarized below:

- Shannon-Wiener: $H' = \sum_{i=1}^{s} (p_i)(\log_2 p_i)$

where *s* is the number of species and *Pi* is the proportion of total samples belonging to *i*-th species.

- Evenness: SHEI= H'/ln (S)

where H' is the value of Shannon-Wiener Index, and s is the number of species in sample (simple species diversity).

Table 1 . Physical parameters and site descriptions of the localities in the study area.

Localities	Physical parameters[a]				Site description	
	Alt (m)	MSRH (%)	MST (°C)	MSP (mm)	Rural	Domestic
K.MARAS	568	62	16.7	723	Bushes, *Quercus* and *Pinus* forests, entisols, semi-humid, caves, pelagic limestones	Briquette, cement and adobe houses, animal barns, food stores
NIGDE	1300	55	11.1	349	Steppe, *Pinus nigra* forests, aridisol, mollisol, semi-arid, highland, caves, tuffs	Briquette, cement, stone and adobe houses, animal barns, food stores, apple farms
KAYSERI	1054	64	10.8	366	*Astragalus, Quercus, Pinus* forests, rodent burrows, alluvial soil, volcanic facies, semi-arid, highland	Briquette, cement and adobe houses, animal barns, chicken houses
ADANA	23	66	18.7	637	Fertile, agricultural area, *Pinus, Abies* forests, alluvial soil, mollisol, humid, lowland	Briquette, cement, stone and adobe houses, animal barns, food stores, citrus farms, cotton fields, chicken farms
OSMANIYE	118	67	19.6	761	Agricultural area, bushes, *Pinus* forests, inceptisols, vertisols, serpantinite, humid, lowland	Briquette, cement, stone and adobe houses, animal barns, food stores, citrus farms, cotton fields, chicken farms
HATAY	85	69	18.2	1173	*Laurus*, *Quercus, Pinus* forests, entisols, semi-humid, Amanos mountains, caves, peridodite	Briquette, cement and stone houses, animal barns, food stores, citrus farms, cotton fields, banana farms
KONYA	1016	60	11.5	324	Steppe vegetation, rodent burrows, agricultural area, mollisols, aridisols, semi-arid, plato, caves, calstic and carbonate rocks	Briquette, cement, stone and adobe houses, animal barns, food stores, corn and wheat fields

[a]Alt: Altitude; MSRH: Mean Seasonal Relative Humidity; MST: Mean Seasonal Temperature; MSP: Mean Seasonal Precipitation. The temperature and relative humidity were calculated as means during the sand fly season, April to October.

Table 2. Number (N) and relative abundance (%) of sand fly species collected in different provinces of the study area between September 2003 and September 2005.

Taxon			No. and (%) of sand flies collected in: [a]																												Total			
			HATAY				OSMANIYE			ADANA				K.MARAS				NIGDE				KAYSERI				KONYA								
		No. of localities[c]	26				6			24				17				6				4				7						90			
Genus	Subgenus	Species	*N*	♂♂	♀♀	%[b]	*N*	♂♂	%♀♀	*N*	♂♂	♀♀	%	*N*	♂♂	♀♀	%	*N*	♂♂	♀♀	%	*N*	♂♂	♀♀	%	*N*	♂♂	♀♀	%	*N*	♂♂	♀♀	%
Phlebotomus	*Phlebotomus*	*papatasi*	233	116	117	28.5	1	0	0.91	179	86	93	16.4	38	16	22	6.2	93	62	31	31.5	0	0	0	0.0	41	23	18	16.5	585	303	282	18.6
	Paraphlebotomus	*sergenti*	77	47	30	9.6	5	2	4.83	32	27	5	3.0	30	16	14	4.9	4	2	2	1.4	0	0	0	0.0	12	7	5	4.8	160	101	59	5.0
		jacusieli	4	2	2	0.1	0	0	0.00	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	4	2	2	0.01
		alexandri	6	5	1	0.7	0	0	0.00	4	1	3	0.05	0	0	0	0.0	1	0	1	0.04	0	0	0	0.0	0	0	0	0.0	11	6	5	0.04
		similis	0	0	0	0.0	0	0	0.00	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	11	6	5	4.5	11	6	5	0.04
	Larroussius	*tobbi*	205	53	152	25.4	18	5	17.213	779	509	270	71.3	29	16	13	4.8	1	0	1	0.04	0	0	0	0.0	0	0	0	0.0	1032	583	449	32.5
		syriacus	26	16	10	3.2	0	0	0.00	17	13	4	1.7	30	24	6	4.9	2	0	2	0.08	0	0	0	0.0	0	0	0	0.0	75	53	22	2.4
		neglectus	25	22	3	3.2	2	2	1.90	33	29	4	3.1	6	5	1	0.1	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	66	58	8	2.1
		perfiliewi	2	2	0	0.03	0	0	0.00	1	0	1	0.02	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	3	2	1	0.01
		galilaeus	89	24	65	11.0	0	0	0.00	18	10	8	1.7	60	2	58	9.8	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	167	36	131	5.3
		transcaucasiacus	54	29	25	6.6	0	0	0.00	9	5	4	0.09	324	11	313	53.1	2	0	2	0.08	0	0	0	0.0	32	0	32	13.0	421	45	376	13.3
	Adlerius	*simici*	6	6	0	0.7	0	0	0.00	2	2	0	0.02	18	12	6	3.0	2	1	1	0.08	0	0	0	0.0	26	26	0	10.5	54	47	7	1.7
		halepensis	30	19	11	3.8	4	3	3.81	1	1	0	0.02	1	1	0	0.02	190	160	30	66.2	6	5	1	100	126	126	0	50.9	358	315	43	11.3
		brevis	0	0	0	0.0	0	0	0.00	0	0	0	0.0	2	2	0	0.04	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	2	2	0	0.01
Sergentomyia	*Sergentomyia*	*theodori*	18	7	11	2.3	57	37	54.320	8	3	5	0.08	22	13	9	3.6	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	105	60	45	3.3
		dentata	40	11	29	4.9	18	13	17.15	9	3	6	0.09	51	9	42	8.4	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	118	36	82	3.7
	Total		815	359	456	100	105	62	10043	1092	689	403	100	611	127	484	100	295	225	70	100	6	5	1	100	248	188	60	100	3172	1655	1517	100

[a]Total number of specimens collected by CDC light traps and indoor aspirators from all sampling stations.
[b]Relative abundance: nx/N*100, nx: number of individuals belonging to species x, N: total number of sampled individuals.
[c]Number of localities with sampling stations in each province.

Table 3. The Shannon-Weiner diversity index (H), evenness (E) and richness (S) for the sand fly species in different provinces and at different altitude ranges of the study area.

Provinces	*H*	*E*	*S*
HATAY	2.03	0.54	14
OSMANIYE	1.32	0.53	7
ADANA	1.03	0.21	13
K.MARAS	1.68	0.44	12
NGD-KYSR	0.84	0.29	8
KONYA	1.42	0.69	6
Altitudes (m)			
0-200	1.52	0.35	13
200-400	1.94	0.63	11
400-600	2.05	0.65	12

RESULTS

A total of 3,172 sand flies (1,655 males and 1,517 females) was collected from domestic and rural sites of seven provinces that were distributed throughout the study area (Table 2). Sixteen species were identified belonging to two genera, 14 *Phlebotomus* and two *Sergentomyia.* The most common species was *Phlebotomus tobbi* Adler & Theodor, accounting for 32.5% of all the flies collected, followed by *P. papatasi* Scopoli (18.6%), *P. transcaucasiacus* Perfiliew (13.3%), *P. halepensis* Theodor (11.3%), *P. galilaeus* Theodor (5.3%), and *P. sergenti* Parrot (5.0%) as the most prevalent species. The remaining species identified were less abundant, with *Sergentomyia dentata* Sinton (3.7%), *S. theodori* Parrot (3.3%), *P. syriacus* Adler & Theodor (2.4%), *P. neglectus* Leger & Theodor (2.1%), *P. simici* Nitzulescu (1.7%), *P. alexandri* Sinton (0.04%), *P. similis* Perfiliew (0.04%), *P. jacusieli* Theodor (0.01%), and *P. perfiliewi* Parrot (0.01%). Only two males were identified as *P. brevis* Theodor & Mesghali in K. Maras province. Of the above species, *P. halepensis, P. galilaeus,* and *P. perfiliewi* from Hatay province, *P. galilaeus, P. transcaucasicus,* and *P. neglectus* from Adana province, and *P. transcaucasiacus, P. sergenti, P. simici,* and *P. halepensis* from Konya province were recorded for the first time in their respective provinces.

Species composition differed from one locality to another and may reflect the changes in the environment of the large-scale area. Overall, while *P. halepensis* were collected from all provinces, *P. papatasi* and *P. sergenti* were found to be two of the most widely dispersing species (Table 2). Members of the subgenus *Larroussius* were predominant (96%) in the Mediterranean sites in the south, while *Adlerius* species such as *P. simici, P. halepensis,* and *P. brevis* were present mostly along the Taurus ridges on the northern border of the study area. Hatay, Adana, and K.Maras provinces are considered as transitional localities since they are influenced by at least three bioclimatic zones (Figure 1, Table 2). The sand fly fauna in these provinces was very rich and often included known species from mountainous areas such as *P. transcaucasiacus* as well as Mediterranean species such as *P. tobbi. P. similis* was only found in Konya province together with the closely related *P. sergenti*. There were differences in the species diversity, as indicated by the values of Shannon-Wiener index (*H'),* and evenness and richness of the sand fly fauna among the provinces (Table 3). Because of its richness of different geographical formations and habitats, all of three parameters were found to be maximal in Hatay province, namely diversity 2.03, evenness 0.54, and richness 14. The relatively low evenness (0.21) and diversity (1.03), and the high richness (13 species) in the Adana province may be due to the dominance of *P. tobbi* (71.3%). Although it is a mountainous area, the diversity index (1.68) was relatively higher, despite low evenness (0.44), in K.Maras than in that of other elevated provinces on Taurus Mountains such as Nigde-Kayseri (0.84). K. Maras province has greater species diversity compared to the other cities because it possesses a gap for sand fly passage through the Cukurova Plain (Figure 1).

The altitudinal distribution and abundance of all the species collected from the study area are shown in Table 4. A majority (64.3%) of all specimens was collected at an altitude range of 0-400 m, but there were three species that inhabited mostly higher altitudes. *P. similis* was only present at 1,000-1,400 m, *P. trascaucasiacus* was found at 1,200-1,400 m, and *P. halepensis* at 1,200-1,600 m (Table 4). The most important *Phlebotomus* vectors of cutaneous leishmaniasis, *P. sergenti* and *P. papatasi,* were collected at all altitudes and their distribution showed a low positive correlation with altitude (r = 0.11 and r = 0.15, respectively). Their highest densities were registered at 0-200 m altitudes. In contrast, the distribution of *P. halepensis* was positively correlated with altitude (r = 0.46). This species was present at very low densities at the lowest altitudes, was absent at altitudes between 600 and 1,000 m and reached a peak at 1,400-1,600 m. *P. tobbi* showed a negative correlation with the altitude (r = -0.78). Densities of this species were very important in the plain (0-200 m); it was almost absent above 400 m altitude.

Between the altitudes of 200 and 400 m, species diversity was more significant (Table 3). The richness, diversity, and evenness were maximal between 200-400 m (H'=1.94; E=0.63; S=11) and 400-600 m (H'=2.05; E=0.65; S=12). Although the highest richness was found at 0-200 m altitude (13), diversity and especially evenness was relatively low. Even though diversity (1.80) and evenness (0.60) were low, the richness was high (10), the altitude range of 1,000-1,200 m being the most critical elevation for dispersing local populations. Evaluation of the results from community analysis suggested two altitudinal assemblages directly associated with sand fly fauna, one at relatively higher altitudes between 1,000 and 1,600 m and one at lower altitudes of 200 to 600 m. The transition zone between these two assemblages is about the range of 800-1,000 m altitude.

DISCUSSION

Phlebotomine sand flies are abundant and widespread in southern Anatolia with at least sixteen species present in the area. More importantly, all proven or suspected vectors of leishmaniasis in Turkey are also present. Because it is the

Table 4. Number (N) and relative abundance (%) of sand fly species collected at different altitude ranges of the study area.

Species	Altitudinal ranges (m)																
	0-199		200-399		400-599		600-799		800-999		1000-1199		1200-1399		1400-1600		
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*
P. papatasi	396	67.7	31	5.3	22	3.8	33	5.6	8	1.4	54	9.2	37	6.3	4	0.7	585
P. sergenti	113	70.6	13	8.1	7	4.4	1	0.6	1	0.6	2	1.3	9	5.6	14	8.8	160
P. jacusieli	0	0.0	2	50.0	2	50.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4
P. alexandri	2	18.2	4	36.4	3	27.3	1	9.1	0	0.0	0	0.0	0	0.0	1	9.1	11
P. similis	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	45.5	6	54.6	0	0.0	11
P. tobbi	935	90.6	60	5.8	4	0.4	9	0.9	9	0.9	12	1.2	2	0.2	1	0.1	1032
P. syriacus	28	37.3	22	29.3	14	18.7	3	4.0	0	0.0	0	0.0	0	0.0	8	10.7	75
P. neglectus	41	62.1	18	27.3	1	1.5	2	3.0	0	0.0	3	4.5	1	1.5	0	0.0	66
P. perfiliewi	3	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3
P. galilaeus	106	63.5	0	0.0	5	3.0	0	0.0	5	3.0	0	0.0	51	30.5	0	0.0	167
P. transcaucasiacus	61	14.5	2	0.5	0	0.0	0	0.0	6	1.4	41	9.7	300	71.3	11	2.6	421
P. simici	7	13.0	0	0.0	3	5.6	0	0.0	3	5.6	24	44.4	17	31.5	0	0.0	54
P. halepensis	32	8.9	5	1.4	10	2.8	0	0.0	0	0.0	64	17.9	46	12.8	201	56.1	358
P. brevis	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	100.0	2
S. theodori	17	16.2	75	71.4	1	1.0	0	0.0	6	5.7	6	5.7	0	0.0	0	0.0	105
S. dentata	40	33.9	27	22.9	1	0.8	2	1.7	40	33.9	5	4.2	2	1.7	1	0.8	118
Total	1,781	56.1	259	8.2	73	2.3	51	1.6	78	2.5	216	6.8	471	14.8	243	7.7	3172

first large-scale study to include nine provinces and their rivers, mountains, and plains, we have obtained information about the geographical distribution of sand fly species and the direct or indirect effects of geographical barriers to this distribution in terms of allopatry. Of the sixteen species we reported, fourteen of them belong to the genus *Phlebotomus* while the remaining two represent *Sergentomyia*. The species of *Phlebotomus* accounted for 93% of the entire collection (3,172) with only 223 (7%) species from the genus *Sergentomyia*. This indicates that for both species diversity and population densities, the *Phlebotomus* species encountered were in relatively large numbers in comparison with members of *Sergentomyia*. Many researchers (Alptekin et al. 1999, Volf et al. 2002, Yaman and Ozbel 2004, Toprak and Ozer 2005) have commented on the paucity of *Sergentomyia* species in south Anatolia. They state that there are some species of *Sergentomyia* in the west and north but remarkably few in southern Anatolia. It is interesting to note that the two *Sergentomyia* species, *S. theodori* and *S. dentata*, which seem to have different environmental affinities do occur together in the Osmaniye and Adana provinces. In contrast to the distribution of the *Sergentomyia* species, those of the *Phlebotomus* species generally embrace all the bioclimatic zones in southern Anatolia.

The distribution of the phlebotomine sand flies is highly disjunctive within its range, depending on local environmental factors such as precipitation and temperature, physical factors such as geographical barriers and habitat availability, and biotic factors such as the distribution and abundance of vertebrate hosts (Cross et al. 1996, Ghosh et al. 1999). Although altitude *per se* is not a selective factor, biotic and abiotic properties of the environment are highly correlated with altitudinal gradients, most obvious of which is climate (Karan et al. 2000). In large parts of southern Anatolia, the climate is characteristic of much of the Mediterranean coast, with a mean temperature of 18-23° C, high relative humidity, and occasional summer showers that permit large populations of adult sand flies to develop. Moreover, geographical and ecological richness of the region provide numerous resting, and perhaps breeding, sites for the flies. Some 14 out of 16 species collected from the study area are present in Hatay Province, making it the most versatile of all the sites. Although Adana and Hatay provinces are located at lower altitudes, they seem to have the typical high altitude species, like *P. syriacus* and *P. transcaucasiacus.* This diversity may reflect the rocky nature and the warm, humid conditions of these provinces combined with the influence of both the Mediterranean and subtropical southeastern elements. K. Maras Province also exhibited a rich sand fly fauna including 12 species. This province is a transitional site that is impacted by the two zones, apparent from the collection from the same site of the species in the Cukurova Plain and the mountainous area in the north. The mountains represent interesting sites in terms of the discrete division observed in the distribution of sand fly species in domestic and rural habitats where most of the *Phlebotomus* species were restricted to domestic and slightly rural habitats. This "patchy" distribution of adults is typical for most of the sand fly species because of their poor dispersal ability (Munstermann et al. 1998). On the other hand, we have found that transitional gaps occur (Figure 1) between western and eastern parts of Hatay Province on the south (GAP 1), between Adana Province and K. Maras Province north of the Amanos Mountains (GAP 2), and between Adana Province and Nigde-Konya Provinces (GAP 3). They contribute to the large-scale horizontal distribution of some species such as *P. sergenti, P. papatasi, P. tobbi,* and *P. transcaucasiacus* throughout the study area. In fact, morphometric and molecular studies on differences among local populations collected from study area confirm this result (unpublished data).

Contrary to the results of Depaquit et al. (2002), we found that two of the most important vector species, *P. sergenti* and *P. similis,* were both present in the sampling sites of the Sarikoy, Beysehir, Aydinkisla, and Sille in Konya Province at the range of 1,132-1,385 m altitude (Table 2). Depaquit et al. (2002) hypothesized that these two species are allopatric at the present time in different countries of the Old World, including Turkey. Turkey is the only country in which both species are present, but *P. similis* is probably present only in its western part and *P. sergenti* only in the eastern part. To confirm if the samples collected from the west side of the West Taurus ridges do not belong to the same species, genomic DNA was extracted from each single sample collected and the ribosomal RNA ITS2 regions were sequenced (Figure 2). The result of molecular study confirms that the specimens are separated into two groups belonging to two different species, *P. sergenti* and *P. similis.* It indicates that *P. sergenti* is a widely distributed species throughout the study area and that these two closely related species are sympatric in Konya Province.

There was a remarkable difference in the diversity of the sand fly fauna, not only among provinces but also altitudes. Like most ectotherms, distribution of sand fly species is heavily dependent on temperature, therefore species situated along altitudinal gradients have to adapt to a variety of climatic conditions (Telfer and Hassall 1999). Temperature is also one of the main factors preventing the spread of both visceral and cutaneous leishmaniasis (Kuhn 1999). This ecological factor varies with the altitude according to the thermal altitudinal gradient (-0.6° C per 100 m). The possible relationship between leishmaniasis transmission and altitude may be closely related to many factors, such as the temperature suitable for the evaluation of *Leishmania* in sand flies (Rioux et al. 1985). This study shows that altitude has an influence upon the spatial distribution of sand flies. Two associations of sand fly faunas were determined in the study area, the first one at a lower altitude (mean 350 m) and the second at a higher altitude (mean 1,300 m). Species diversity was relatively high at about 900 m, corresponding to the transition between the lowland and the mountain. Guernaoui et al. (2006) found similar results about altitudinal structuring of sand fly species in the High-Atlas Mountains, Morocco. Two other associations were fixed among the provinces in terms of horizontal distribution of sand flies, the first one among

Hatay, Adana, and K.Maras and the second one among Konya, Adana, and Nigde-Kayseri provinces. The species richness and diversity were the highest in Cukurova Plain consisting of Adana, Osmaniye, and the western Amanos Mountains in Hatay Province that corresponds to the horizontal transition between the eastern and western parts of the study area.

The abundance of a sand fly species is not by itself sufficient to incriminate it as a vector. Some populations of suspected vectors appear to be small, while others seem to have too short a season to maintain the circulation of a *Leishmania* (Killick-Kendrick 1990). *P. sergenti* is one of the proven vectors of *L. tropica* in several countries around the Mediterranean (Jacobson et al. 2003). In contrast to previous studies (Volf et al. 2002, Yaman and Ozbel 2004), this species showed widespread distribution throughout the study area but with small populations. The species represented 5% of all the *Phlebotomus* caught. *P. sergenti* has been described as a "mountain" species (Seyedi Rashti and Nadim 1992) that is most common at 500-700 m above sea level (Büttiker and Lewis 1983). In this study, it was found to be more abundant (70.6%) between 0 and 200 m, but no significant correlation was found between its density and altitude. Yaman and Ozbel (2004) indicated that this species was only common at lower altitudes and was relatively rare in the mountains in Hatay Province of Turkey. Furthermore, even if this vector prefers the semi-arid bioclimate, we collected it in all bioclimatic belts in the study area. According to Guernaoui et al. (2006), *P. sergenti* is largely widespread in the whole of Morocco. The distribution pattern of *P. sergenti* corresponds with that of ACL and could increase the risk of spreading *L. tropica* and also *L. infantum* from southeastern Anatolia focus to Konya Province and other nonendemic sites in Turkey. Three *P. sergenti* females were found to be positive for the parasite *L. tropica* in Alibozlu village in Adana as a new focus (Pazarbasi et al. 2006).

The most abundant species in the study area, *P. tobbi,* was frequently collected from the lowlands, Hatay and Adana, at the range of 0-400 m altitude (96.4%). Seyedi-Rashti and Nadim (1992) described this species as rare in mountainous areas. *P. tobbi* has been previously reported from Hatay Province with a small population (Yaman and Ozbel 2004). *P. tobbi* is a member of the subgenus *Larroussius,* which includes all the vectors of the parasites causing human VL (Killick-Kendrick 1990). The result of recent studies in Cyprus implicates *P. tobbi* as a vector of VL in the eastern part of the Mediterranean basin, where *P. neglectus* is absent (Leger et al. 2000). Ertabaklar et al. (2005) found that two *Phlebotomus* species, *P. transcausasiacus* and *P. neglectus,* together with *P. tobbi* belonging to *Larroussius* subgenus, constitute potential vectors of human leishmaniasis in the Corum Province in northern Turkey. Serin et al. (2005) analyzed ten microcapillary cultivated isolates from cutaneous cases and five microcapillary cultivated isolates from visceral cases by polymerase chain reaction-RFPL in Adana Province. Of ten isolates, three were genotyped as *L. infantum* and seven of ten isolates from the microcapillary cultivated cutaneous cases were genotyped as *L. tropica*. Because of frequent human travel between Hatay and Syria, some of these individuals carry Syrian parasites into Hatay (Yaman and Ozbel 2004) and from this province to Adana. The possibility that at least some cases of HCL in Hatay and Adana are also caused by *L. infantum,* perhaps transmitted by *P. (Larroussius) syriacus* and *P. tobbi,* merits exploration.

P. papatasi, as one of the vectors of HCL, is often abundant in areas of steppe and semi-arid zones where temperatures are high but humidity is not extremely low (Belazzoug 1991). This species also showed widespread distribution throughout the study area at the range of 0-600 m altitudes (76.8%). The high occurrence of *P. papatasi* in the lowlands and its low frequency in the mountains could be explained by its preference for the semi-arid areas.

In conclusion, Adana and Hatay provinces have the richest sand fly fauna in southern Anatolia, and these provinces seem to be transitional areas between western and eastern Anatolia. Mountains in the study area did not appear to be important geographical barriers for sand fly distribution. Future investigations are needed to clarify the *L.infantum* transmission risk in this area by isolating and typing *Leishmania* strains from vectors. Another aim of future research in this area will be to make detailed investigations on the biology and ecology of *P.tobbi, P. halepensis, P. syriacus,* and *P. transcaucasiacus.*

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