

# Fauna, Seasonal Activity, and Altitudinal Distribution of Phlebotomine Sand Flies (Diptera: Psychodidae) in Leishmaniasis Endemic Area, Aydın Mountains, and Surroundings in Western Turkey

*Türkiye'nin Batısında, Leishmaniasis'in Endemik Olduğu Aydın Dağları ve Çevresinde Kum Sineği (Diptera: Psychodidae) Faunası, Mevsimsel Aktiviteleri ve Yüksekliğe Göre Dağılımları*

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## ABSTRACT

**Objective:** Human and canine leishmaniasis (CanL) are endemic in Turkey, and cutaneous leishmaniasis (CL) is a primary public health problem among vector-borne diseases, with 1500-2000 autochthonous cases per year. In this study, we aimed to perform a comprehensive entomological survey, including the detection of sand fly fauna, seasonal activity, and altitudinal distribution, together with the ecological features of Aydın Mountains and their surroundings that are endemic areas for CL caused by *Leishmania tropica* and CanL caused by *L. infantum* MON-1.

**Methods:** The survey was carried out from June 2009 to July 2010. Ten localities with different altitudes were selected. Sand fly collection was done every 15 days in July and August when sand flies are most active and every 30 days between September and June (14 months, 16 samplings) using CDC light traps and sticky traps. During the collection, the temperature, humidity, and ecological and environmental features were recorded. Sand fly specimens were dissected and identified at species level using written keys.

**Results:** A total of 6712 sand fly specimens (3268 females and 3444 males) were collected from 10 localities throughout all study periods. Species identification revealed that nine and three species belonging to *Phlebotomus* (*P. major* s.l. 30.38%, *P. tobbi* 22.93%, *P. papatasi* 5.88%, *P. sergenti* s.l. 4.51%, *P. alexandri* 4.26%, *P. simici* 3.50%, *P. burneyi* 0.63%, *P. brevis* 0.45%, and *Transphlebotomus* spp. 0.28%) and *Sergentomyia* (*S. dentata* 23.17%, *S. minuta* 2.43%, and *S. antennata* 1.58%) were found, respectively. The seasonal activities of these 12 sand fly species were determined, and *P. major* s.l. and *P. tobbi*, which were probable vectors of VL and CanL, were present in the study area. *P. sergenti* s.l., a probable vector of CL, was also found in the region and was active for seven months between April and October. *P. sergenti* s.l. was found in the 400-600 m altitude in July, while *P. major* and *P. tobbi* were found in high densities in the 200-400 m altitude in September.

**Conclusion:** Our results showed that leishmaniasis vectors are present in different altitudes in the Aydın mountains and surroundings. The findings revealed the time intervals of parasite transmission in the area and the period of applying protective measures, such as insecticide application.

**Keywords:** Sandy fly, seasonal activity, leishmaniasis, Aydın Mountains, Western Anatolia



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## ÖZ

**Amaç:** İnsan ve kanin leishmaniasis (CanL) açısından endemik bir ülke olan Türkiye’de, kutanöz leishmaniasis (KL) yılda 1500-2000 yerli olgu ile vektör kaynaklı hastalıklar arasında birincil halk sağlığı sorunudur. Bu çalışmada, *Leishmania tropica*’nın neden olduğu CL ve *L. infantum* MON-1’in neden olduğu CanL bakımından endemik bir alan olan Aydın Dağları ve çevresinde bulunan kum sineğinin faunası, mevsimsel aktiviteleri ve yüksekliğe göre dağılımları ile ekolojik özelliklerinin de belirlenmesini içeren kapsamlı bir entomolojik araştırma amaçlanmıştır.

**Yöntemler:** Araştırma, Haziran 2009 ile Temmuz 2010 tarihleri arasında gerçekleştirilmiştir. Farklı yükseklik aralıklarına göre toplam 10 lokasyon seçilmiş, CDC ışık tuzakları ve yapışkan tuzaklar kullanılarak kum sineklerinin en aktif olduğu Temmuz ve Ağustos aylarında 15 günde bir, Eylül ve Haziran ayları arasında 30 günde bir olmak üzere kum sineği örnekleri toplanmıştır (toplam 14 ay, 16 örnekleme). Örnek toplama sırasında sıcaklık, nem, ekolojik ve çevresel özellikler kaydedilmiştir. Kum sineği örnekleri, teşhis anahtarları kullanılarak tür düzeyinde tanımlanmıştır.

**Bulgular:** Tüm örnekleme periyotları boyunca, 10 lokasyondan toplam 6712 kum sineği örneği (3268 dişi ve 3444 erkek) toplanmıştır. Morfolojik tür teşhisinde *Phlebotomus* cinsine ait dokuz (*P. major* s.l. %30,38; *P. tobbi* %22,93; *P. papatasi* %5,88; *P. sergenti* s.l. %4,51; *P. alexandri* %4,26; *P. simici* %3,50; *P. burneyi* %0,63; *P. brevis* %0,45; *Transphlebotomus* spp. %0,28) ve *Sergentomyia* cinsine ait üç tür (*S. dentata* %23,17; *S. minuta* %2,43; *S. antennata* %1,58) bulunmuştur. On iki kum sineği türünün mevsimsel aktivitesi belirlenmiş, VL ve CanL’nin olası vektörleri olan *P. major* s.l. ve *P. tobbi* ile CL’nin olası vektörü olan *P. sergenti* s.l. de çalışma alanında bulunmuş olup, Nisan ile Ekim ayları arasında yedi ay boyunca aktivite gösterdikleri saptanmıştır. *P. sergenti* s.l.’nin Temmuz ayında 400-600 m’de, *P. major* ve *P. tobbi*’nin ise Eylül ayında 200-400 m yükseklik aralığında en fazla yoğunluğa sahip olduğu tespit edilmiştir.

**Sonuç:** Sonuçlar, Aydın Dağları ve çevresinde leishmaniasis vektörlerinin farklı yüksekliklerde bulunduğunu göstermiştir. Elde edilen bulgular, çalışma alanında parazitin bulaşma dönemi ve insektisit uygulaması gibi koruyucu önlemlerin uygulama periyodunu ortaya koymuştur.

**Anahtar Kelimeler:** Kum sineği, mevsimsel aktivite, leishmaniasis, Aydın Dağları, Batı Anadolu

## INTRODUCTION

Phlebotominae sand flies play a role in the transmission of different *Leishmania* species that cause leishmaniasis, as well as bacteria (*Bartonella bacilliformis*) and various phleboviruses. In addition, sand fly bites can cause allergic reactions in sensitive people (1-6). More than one thousand species of sand flies have been identified in the New and Old World, but only 10% of them are proven or probable vectors of the *Leishmania* species in mammals including humans (7-9). The species belonging to *Phlebotomus* genus (Diptera: Phlebotomidae) are very important because they are the biological vectors of different *Leishmania* species and phleboviruses in the Old-World countries including Turkey (10).

All clinical forms of leishmaniasis are still one of the most neglected diseases of the world and generally affect developing countries. Two million cases occur every year and 350 million people in the world are at risk of infection according to World Health Organization reports. Over the past decade, scientific approaches have been developed in the diagnosis, treatment and prevention measures against leishmaniasis to facilitate sustainable implementation of national and regional control programs (11). To date, no vaccines or drugs have been developed to provide long-term protection and effective immunity human against leishmaniasis (12). Therefore, vector control in integrated control programs has gained great importance for fighting these diseases (13,14).

Turkey is a bridge between Europe and Asia/Middle East and has different ecological and climatic features. Zoonotic cutaneous leishmaniasis (ZCL), anthroponotic cutaneous leishmaniasis (ACL) and zoonotic visceral leishmaniasis (ZVL) in humans, and canine leishmaniasis (CanL) in dogs are commonly seen in Turkey (15).

Understanding the population dynamics and ecological characteristics of *Phlebotomus* species, their vectorial capacity in the particular areas where the disease is endemic, and the epidemiological features are important for the development of integrated vector control strategies (16). Therefore, entomological studies performed in different regions of Turkey are mainly in the areas where visceral and/or cutaneous leishmaniasis is endemic.

Although ACL is often seen in southeastern Turkey and the eastern part of the Mediterranean, it is mainly reported from Aydın province in the western part of Turkey. Additionally, CanL cases have also been reported from Aydın and its surroundings since 1993. It is thought that the province has increasing importance in terms of public and veterinary health (17,18). Therefore, the aims of this study were to determine the sand fly fauna, diversity and density of the species according to the altitudinal range and climatic factors, and to present the findings of the seasonal monitoring.

## METHODS

### Study Area and Sand Fly Collections

Aydın province (37.872208 E, 27.919010 N) located in Western Turkey has around one million inhabitants and surrounded by mountains in the South and North. Large fertile plains are located in the central and western parts of the province (Figure 1). The province experiences Mediterranean climate with hot and dry summers while winters are warm and rainy. The highest temperature is between 41.2 °C and 42.1 °C while the average temperature is between 27.2 °C and 28.7 °C in June and July. The average relative humidity is around 61% in Aydın province. The study was carried out in a transect from Aydın plain to the top of Aydın mountains, providing an altitudinal range between 65 and 1891 meters a.s.l.

The sand fly sampling was performed between June 16, 2009 and July 31, 2010 in every 15 days in July and August when sand flies are mostly active and every 30 days for the rest of the months using miniature CDC light traps (LTs) and sticky traps (STs). A total of 16 collections were done during the study period.

Although ACL and CanL have been reported from almost all settlements of the province (17), 10 villages were randomly selected for sand fly sampling on or around Aydın mountains according to the altitudinal range (two in each 200 m altitude range) (Figure 1). For setting up two CDC LTs, one closed points such as animal barns, poultrys, dog shelters etc., and one open points outside per village were selected. The LTs were set up in the evening (17:00-18:00) at 1-1.5 meters above the ground and picked up early in the morning (06:00-07:00) for one night. In

each sampling time, the LTs were placed in the same point as close as possible. Two LTs were placed in each village at 16 sampling times, and a total of 320 LTs were used during the entire study.

The geographical locations of each collection site were recorded using GPS, and a total seven data logger (HOBO) was placed together with LTs for measuring temperature and humidity in the microenvironmental level and left there for one year. The data loggers were programmed to record temperature/humidity every one hour starting from June 16, 2009. Additionally, other environmental parameters such as the number of animals in the location, physical features of the structure and vegetation were also recorded.

A4 sized regular papers were coated with castor oil to be used as STs and according to the suitability of the location, 10-30 STs per village were placed overnight in outdoor (wall cracks, drainage holes on the walls, etc.) and indoor (barns, houses, etc.) in each collection time. Sand flies were collected from a total of 2240 STs during the study. The STs collected were calculated in square meters to provide standardization while examining the species distribution based on the altitude.

The sand fly specimens were collected from the cages of LTs using a mouth aspirator and from STs using a fine brush dipped in 70% alcohol. After collection the specimens were stored in 96% ethanol for morphological identification. The specimens were individually prepared under the stereomicroscope (Olympus

SZ40) and the head and genitalia were cut, clarified in Marc-Andre solution and mounted in swan solution then examined under the light microscope (Olympus CX31). Specimens were morphologically identified based on male external genitalia and female spermatheca and pharyngeal armature/cibarial armature using the several published written keys for Mediterranean sand flies (10,19-23).

## Data Analysis

The seasonal activity and dominance of sand fly species were calculated on monthly and yearly counts. The temperature and humidity data were extracted from seven data loggers and analyzed at the end of the study. The lowest and highest temperature/humidity values in the entire working time of the data loggers and the averages of temperature/humidity during collection days were evaluated.

Shannon-Wiener species diversity index and Evenness degree were used to estimate species diversity and distribution of individuals among all species to identify species diversity grade as a numerical value and to make statistical comparison of diversity grades in different localities (24).

“Kendal’s Tau b” correlation measure was used to determine the relationship between the seasonality of different species and temperature/humidity. In this test, the correlation coefficient varies between (-1) and (+1). While the positive correlation indicates that the rows of both variables increase together, the negative correlation coefficient means that the number of rows of one variable increase while the number of rows of the other decreases. The correlation coefficient between 0 and 0.49 is interpreted as a weak relationship, a value between 0.50 and 0.69 is a medium relationship, and a value between 0.70 and 1 is interpreted as a high relationship.

## RESULTS

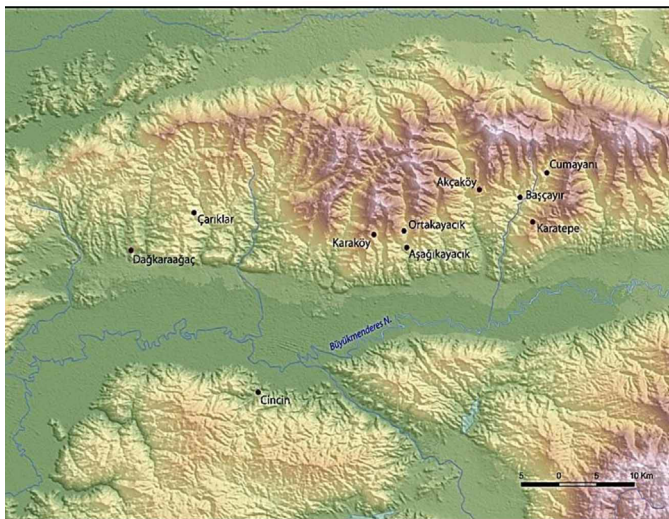
### Sand Fly Fauna

A total of 2.783 and 3.929 sand fly specimens were caught using CDC LTs and STs, respectively. Among these 6.712 specimens, 3.268 were female and 3.444 were male, which makes the female/male ratio as 0.95 during all study periods. Nine and three species belonging to *Phlebotomus* and *Sergentomyia* genera were recorded in the study area, respectively. Among these, *P. major s.l.* was the most abundant species with 2.039 (30.38%) specimens, followed by *S. dentata* (n=1555; 23.17%) and *P. tobbi* (n=1539; 22.93%) (Table 1).

Three species *P. major s.l.*, *P. tobbi* and *P. sergenti s.l.* were recorded from all villages and altitudes in both trapping methods. *P. papatasi*, was the fourth most widespread species but with a smaller number of specimens (Figure 2).

### Seasonal Dynamics of Sand Fly Species

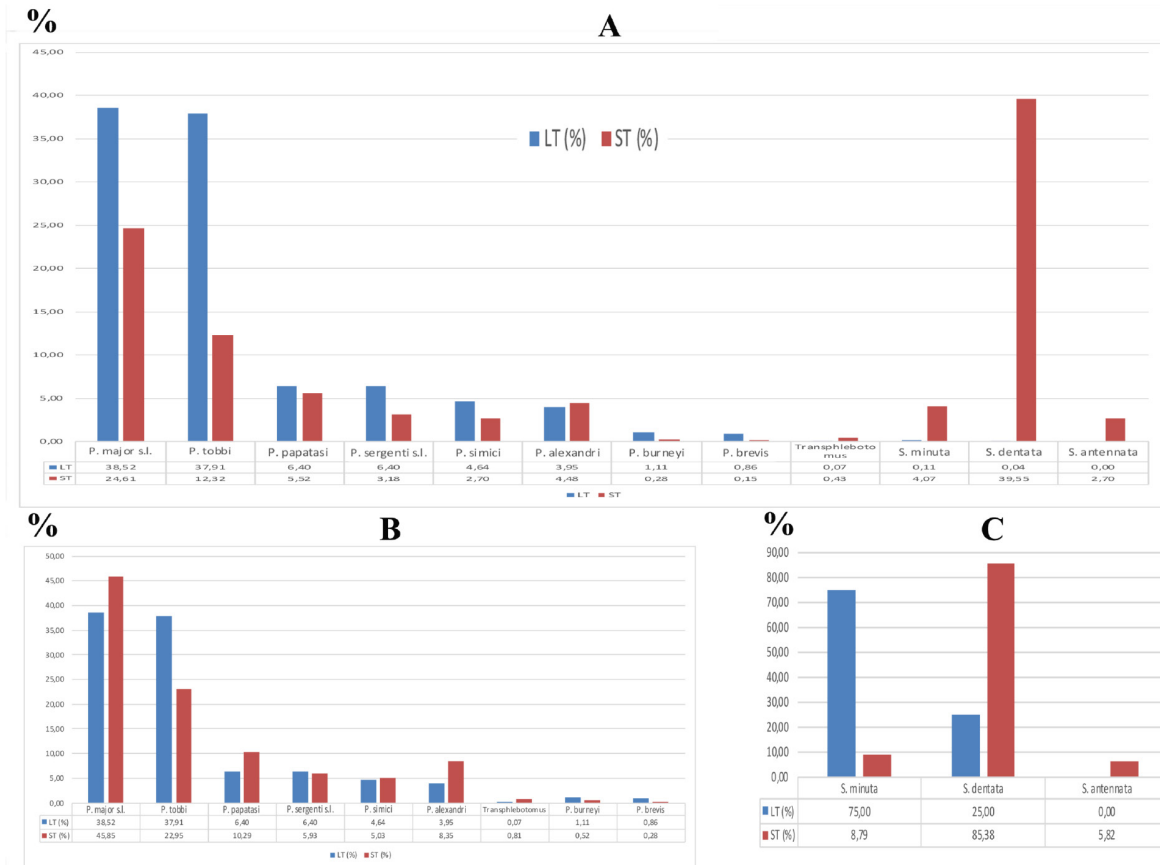
Most of sand fly specimens were collected between June-September. On the other hand, only one specimen (by sticky trap) in November, two specimens in February and one specimen (by CDC light trap) in March were found while no specimens were obtained in December and January. It was observed that April is the time when sand flies first start to emerge. The peak started in June with 982 specimens and continued in July with 1.014 specimens (Figure 3). The analysis of seasonal activities of all sand fly species revealed that only four *Phlebotomus* (*P. alexandri*,



Village (Locality)	Altitude (m)	Altitudinal range (m)	Coordinates Lat/Long
Karatepe	840	800-1000	37 55 47 N / 28 05 12 E
Karaköy	830	800-1000	37 54 55 N / 27 53 49 E
Akçaköy	780	600-800	37 57 59 N / 28 01 23 E
Ortakayacık	655	600-800	37 55 10 N / 27 55 58 E
Cumayanı	574	400-600	37 59 07 N / 28 06 13 E
Çarıklar	454	400-600	37 56 24 N / 27 40 53 E
Aşağıkayacık	376	200-400	37 54 02 N / 27 56 10 E
Başçayır	340	200-400	37 57 27 N / 28 04 18 E
Dağkaraağaç	107	0-200	37 53 51 N / 27 36 23 E
Cincin	75	0-200	37 44 10 N / 27 45 30 E

**Figure 1.** The location of the selected villages for sand fly collection and altitudes





**Figure 2.** Sand fly composition of the study area. **A.** All sand fly species, **B.** *Phlebotomus* species (n=4888); **C.** *Sergentomyia* species (n=1824)  
 LT: Light traps. ST: Sticky traps. Specimens caught using LT (n=2783) and ST (n=3929) were evaluated separately for the graph

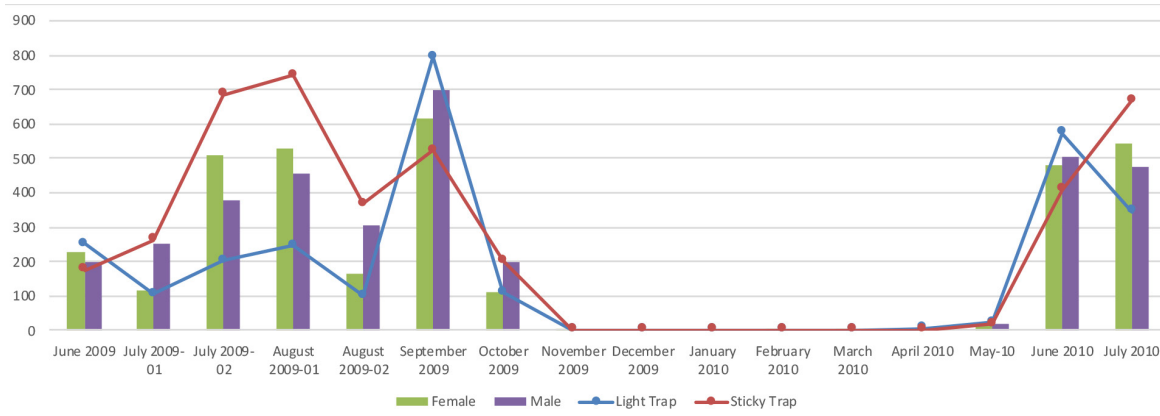
*P. burneyi*, *P. simici*, *P. brevis*) and one *Sergentomyia* (*S. dentata*) species have three peaks while the others have two peaks in the season. The features of seasonal activity of vector sand fly species are given in Table 2 and Figure 4. The seasonal activity of other sand fly species is given as supplemental data (Suppl File 1).

**Altitudinal Distribution**

Seventy percent and 48% of total specimens were caught at an altitudinal range of 200-400 m by CDC LTs and STs, respectively (Table 3). Additionally, around 50% of the specimens were caught at an altitude of 800-1000 m by sticky traps, and 1,552 (40%) and

307 (7.8%) of the specimens were identified as *S. dentata* and *P. major s.l.*, respectively. At the same altitudinal range (800-1000 m), 176 (6.3%) *P. major s.l.* were caught using CDC LTs, however, no *Sergentomyia* specimens were found. All sand fly species were analyzed according to the altitudinal range and the results are given in Suppl File 2.

When the collection locations were evaluated according to altitude groups, statistically no relationship was observed between the number of individuals caught and temperature (Kendall  $\tau$ ,  $p \geq 0.05$ ) and humidity (Kendall  $\tau$ ,  $p \geq 0.05$ ).



**Figure 3.** Seasonal distribution of the total sand fly specimens collected in 16 sampling periods by months between June 2009 and July 2010

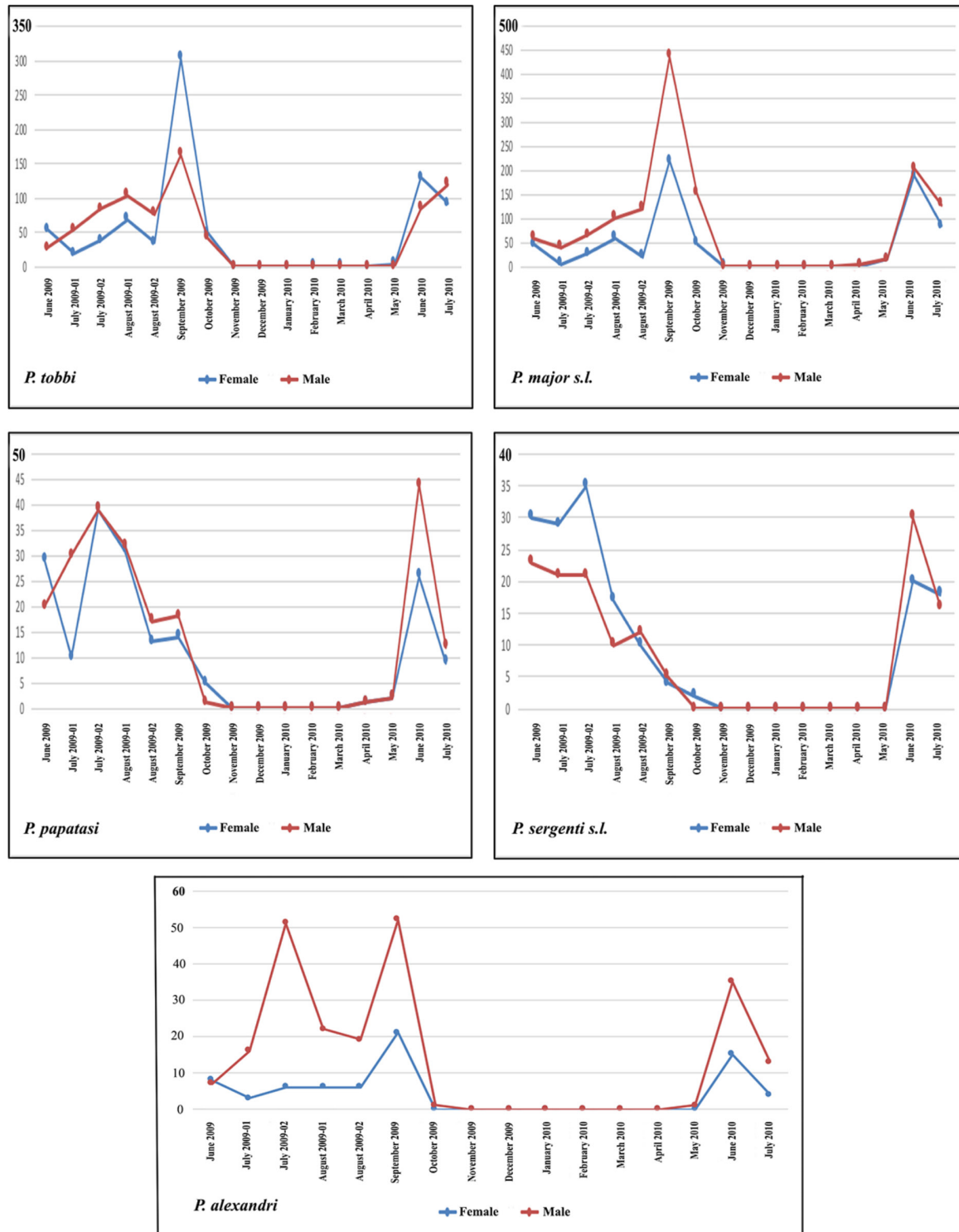
We also investigated whether there was a statistical difference between the number of sand flies caught depending on the altitude, and we observed that there were differences both among sand fly species and in the total number of specimens according to the altitude (Kruskal-Wallis test,  $p < 0.05$ ) (Figure 5, 6).

### Diversity Index

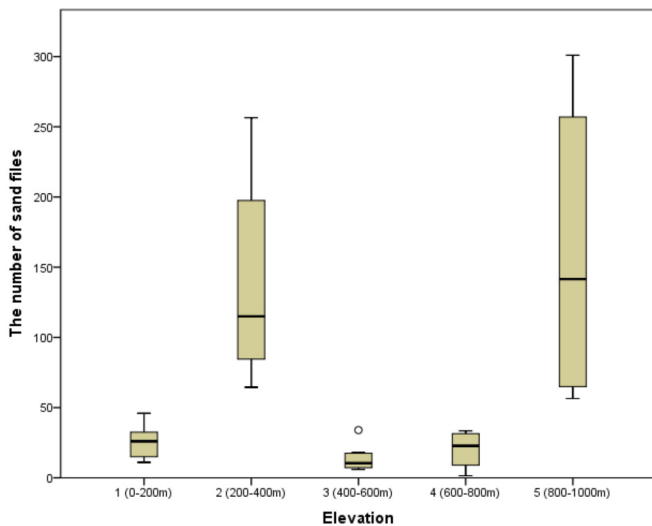
The analyzes of the diversity index showed that *P. sergenti s.l.*, *P. major s.l.* and *P. tobbi* was present in all localities, and *P. sergenti s.l.*

had the highest value according to the Shannon index. The highest species diversity was detected in Ortakayacak (655 m) village (Table 4). In addition, highest species diversity was recorded at the end of July compared to other collection times, according to Shannon index (Table 5).

Species diversity and species richness for the specimens collected by LTs were found high in the range of 600-800 m and 800-1000 m, respectively. On the other hand, species diversity and species



**Figure 4.** The seasonal distribution of five probable vector species between June 2009 and July 2010 in the study area



**Figure 5.** Total number of sand flies caught according to the altitude

richness for the samples collected by STs were found high in the range of 400-600 m and 0-200 m by Shannon index (Table 6).

**The Values Obtained by Data Loggers**

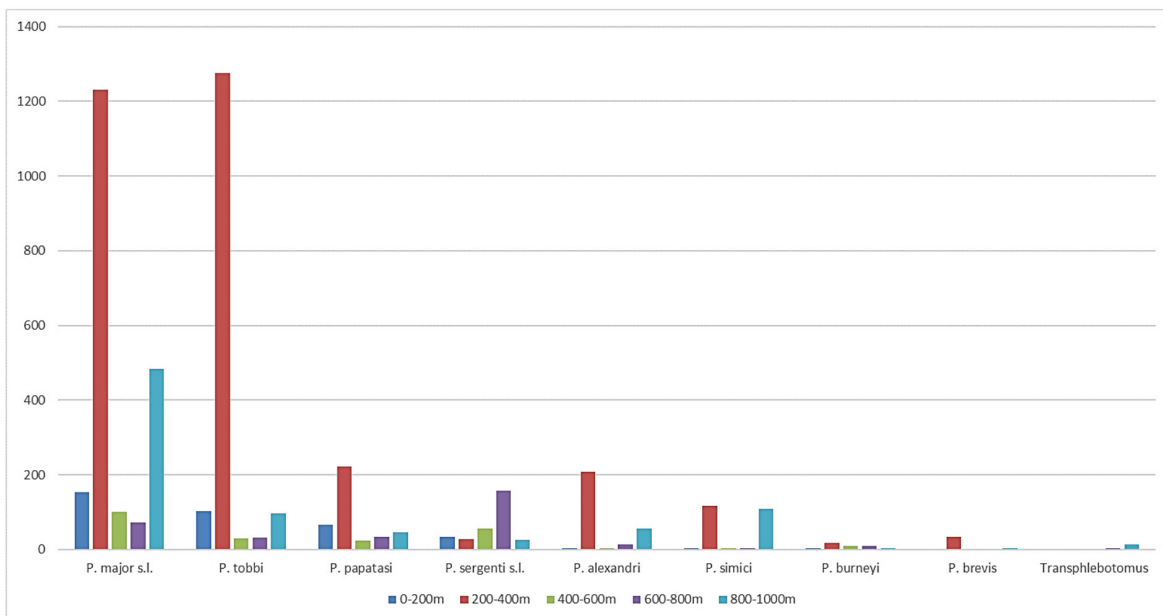
The temperatures were detected as between 16.6 and 20.6 °C (ave 18.7 °C), and RH% were between 54.1-60.9% (ave 58.0%) according to the locations. The values extracted from data loggers showing minimum, maximum and average temperature and humidity in the seven localities were given in Figure 7. According to the data obtained from data loggers, during the months of June-September, between 10:00 and 03:00 at night when sand flies are most active, the average temperature and humidity in localities was 23.1-26.2 °C (min-max) and 43.5-47.7% (min-max) respectively. The temperature and humidity values obtained from the data loggers placed in the localities were also evaluated according to the non-parametric Kendall's Tau b correlation coefficient. A medium positive relationship was found between

the presence of three species [*P. papatasi* (0.52), *P. major s.l.* (0.51), *P. tobbi* (0.51)] and temperature, but other *Phlebotomus* species showed weak positive relationships (>0.49) with temperature. On the other hand, a weak negative relationship (>0.42) was detected between the presence of the species and humidity.

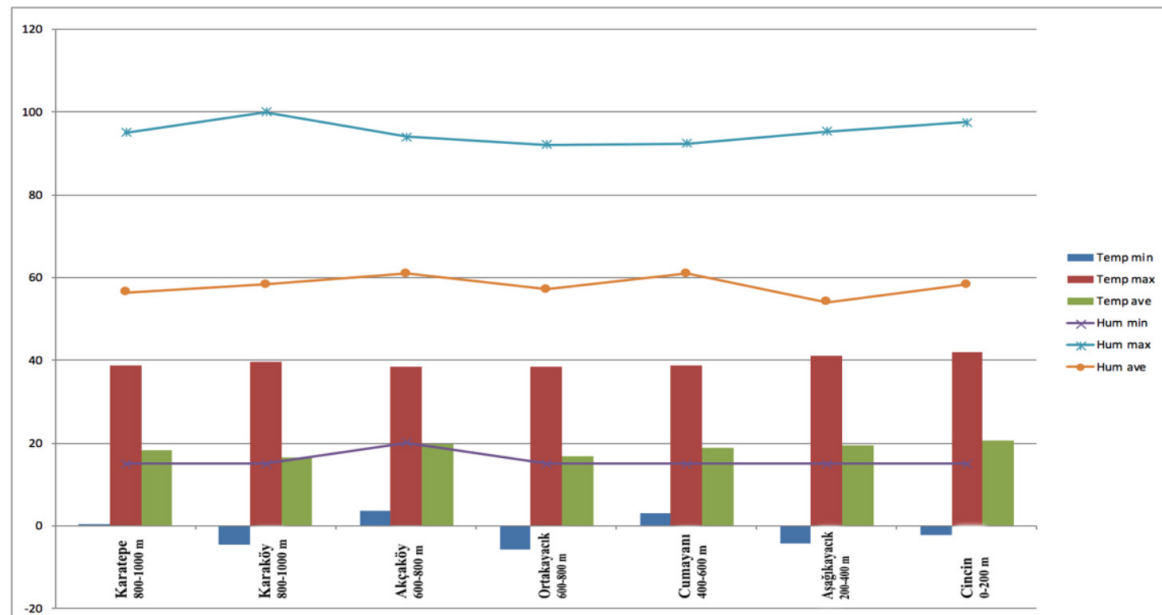
**DISCUSSION**

In the leishmaniasis endemic areas, the detection of sand fly species and their distribution provides essential knowledge to understanding the transmission dynamics of different clinical forms of leishmaniasis. In addition, the data about the seasonal activity of sand fly vectors improves knowledge for better decision making on appropriate time periods for application of vector control measures in different endemic areas. Aydin province and its surroundings, is one of the important endemic areas for ACL and CanL in the western part of Turkey. In the present study, we aimed to provide new data on seasonal activity of sand fly species in the area in relation to altitude and microclimatic data. To this aim, detailed analyses after the morphological identification of sand flies obtained from 16 collections between June 2009 and July 2010 in the selected 10 villages were carried out.

A total of 12 sand fly species (9 *Phlebotomus*, 3 *Sergentomyia*) were identified in the study. The ratios of sand fly specimens belonging to *Phlebotomus* and *Sergentomyia* genera were 72.82% and 27.18%, respectively. *Phlebotomus* specimens were mainly found in the areas where humans and animals live while *Sergentomyia* species were mostly caught by STs in stony areas such as stone walls where many reptiles were observed. In previous studies conducted in Turkey, it is emphasized that the populations of species belonging to the genus *Sergentomyia* are generally less. Although the number of *Sergentomyia* species collected were less than the *Phlebotomus*, the number of individuals was found to be significantly higher than in previous studies (25-28). The reason for this is thought to be due to the researchers placing traps more on the animal barn or areas where people live, and generally using only the LTs. As we applied in the present study, the usage of LTs and STs



**Figure 6.** The distribution of *Phlebotomus* species found in all altitudinal ranges



**Figure 7.** Temperature and humidity data obtained from data loggers

together has also an advantage not only to catch all sand fly species belonging to both genera in the area but also to perform quantitative analyses.

*Phlebotomus tobbi* (22.93%) is the proven vector species of *L. infantum* in the eastern part of the Mediterranean Region of Turkey and *P. major s.l.* (30.38%), a well-known vector species of *L. infantum* in Europe and Turkey, were found as the dominant species. *P. jacusieli*, *P. perfiliewi* and *S. theodori* species, previously found in Aydın province (18), were not encountered in the present study, however, *S. antennata* were not reported in the previous studies. This probably happens due to the morphological similarity of three *Sergentomyia* species present in Turkey. Beside these species, other proven or probable vector species of different *Leishmania* parasites, *P. papatasi*, *P. sergenti s.l.*, and *P. alexandri* (29), were found in the study area. In addition, all species caught during the present study have also been reported previously in the different provinces of Turkey (16,18,26-28,30-33).

Almost 68% and 90% of *P. major s.l.* and *P. tobbi* were collected in the 0-400 m altitudinal range by both types of traps, respectively. Additionally, 24% of *P. major s.l.* specimens were caught in the 800-1000 m altitudinal range. When we checked the ACL cases in Aydın province between 2006 and 2014, we have found that 251 ACL cases were reported in this period and 85.25% (n=214) of them were from rural settlements located in between 40 and 400 meters. Only 16 (6.4%) ACL cases were reported from the settlements located above 600 m in the area (Özbel Y-unpublished data). A previous study analyzing the *Leishmania* isolates from different provinces reported the presence of only *L. tropica* (n=63) in Aydın province (15). Because of this report and altitudinal distribution of ACL cases as mentioned above, we also evaluated our findings related to *P. sergenti s.l.* and we have seen that the vector species of *L. tropica*, *P. sergenti s.l.*, was detected at all altitudes, but half (52%) were found in the 600-800 m range. These previous and present findings emphasize the complication of the situation in the area in terms of leishmaniasis. There is a need to check much more ACL clinical samples (lesion aspiration fluids, biopsies) instead of isolates by molecular methods for species identification

to understand the causative agent(s) in the area clearly. Because of the lack of records about locations of *Leishmania* infected dogs, it is difficult to analyze CanL situation in this endemic area.

Studies related to sand flies carried out in Turkey showed that climatic and microenvironmental specifications together with altitude are main factors affecting sand fly species distribution and abundance (28,34,35). In our study, we also determined that there are differences according to the total number of species and the altitude among the sand fly species caught. In addition, we realized that this difference is not a gradual decrease or increase. We observed that the temperature and humidity did not change significantly according to the locations in different altitudinal ranges (Figure 7). This is also reflected in the species diversity detected in the locations (min 8; max 12 species). For this reason, and probably related to other abiotic factors, such as geographical specifications of the study area, vegetation and soil type, we could not find a strong relationship between temp/hum and species diversity. On the other hand, the population size of the three vector species was significantly higher (Table 1) at the altitude of 200-400 m where the average temperature 19.6 °C and average humidity 54.1%. Therefore, we can conclude that the number of sand flies caught in the study area may be related to the microhabitat conditions (e.g., olive trees in lower and fig trees in higher altitudes) in the environment rather than or together with the altitude.

*P. neglectus* and *P. syriacus*, which are morphologically similar species in *P. major* group, have been reported as separate species in many faunistic studies. It has been stated that *P. syriacus* can be found in the south of Turkey while *P. neglectus* can be found in all geographical regions (36). However, since both of them belong to the *P. major* group and molecular analyzes were not performed in the present study, we preferred to report it as *P. major s.l.* Most of the *Larrousius* species are proven vectors of *L. infantum* in Southern Europe, Southwest Asia and North Africa (29,31). *Phlebotomus neglectus* has been reported as a vector of *L. infantum* causing VL in Greece (37), Croatia (38) and Italy (39). It has also been stated that

**Table 1.** Distribution of sand fly species according to 10 villages of the Aydın Mountains and surroundings

Villages (altitudem)	Trap type	Sand fly species											Total	
		<i>P. major s.l.</i>	<i>P. tobbi</i>	<i>P. papatasi</i>	<i>P. sergenti s.l.</i>	<i>P. alexandri</i>	<i>P. simici</i>	<i>P. burneyi</i>	<i>P. brevis</i>	<i>Transphlebotomus spp.</i>	<i>S. dentata</i>	<i>S. antemata</i>		<i>S. minuta</i>
Cincin (75 m)	LT	8	18	8	2	0	0	2	0	0	0	0	1	39
	ST	59	59	6	27	1	1	1	1	0	0	0	49	204
Dağkaraağaç (107 m)	LT	62	21	19	4	1	0	0	0	0	0	0	1	108
	ST	25	6	34	1	1	2	0	0	0	0	0	1	70
Başçayır (340 m)	LT	557	642	33	7	78	39	11	11	0	1	0	1	1380
	ST	505	261	25	3	111	26	1	2	0	0	0	0	934
Aşağıkayacık (376 m)	LT	137	302	65	9	11	31	4	12	0	0	0	0	571
	ST	31	70	100	10	9	21	1	0	2	0	0	1	245
Çarıklar (454 m)	LT	17	15	4	19	1	0	6	0	0	0	0	0	62
	ST	5	3	16	25	2	1	4	0	0	0	1	0	57
Cumayanı (574 m)	LT	60	11	4	10	0	1	0	1	0	0	0	0	87
	ST	18	1	1	2	1	1	0	0	0	1	0	2	27
Ortakayacık (655 m)	LT	50	15	9	31	10	3	7	0	1	0	0	0	126
	ST	0	1	0	11	0	0	1	0	2	0	0	0	15
Akçaköy (780 m)	LT	5	3	5	80	0	0	1	0	0	0	0	0	94
	ST	17	14	20	36	4	0	0	0	0	1	0	0	92
Karaköy (830 m)	LT	42	6	0	6	0	1	0	0	0	0	0	0	55
	ST	4	0	0	0	0	0	1	0	0	0	0	0	5
Karatepe (840 m)	LT	134	22	31	10	9	54	0	0	1	0	0	0	261
	ST	303	69	15	10	47	54	2	3	13	1552	105	107	2280
Total specimens by trap type	LT	1072	1055	178	178	110	129	31	24	2	1	0	3	2783
	LT %	<b>38.52</b>	<b>37.91</b>	<b>6.40</b>	<b>6.40</b>	<b>3.95</b>	<b>4.64</b>	<b>1.11</b>	<b>0.86</b>	<b>0.07</b>	<b>0.04</b>	<b>0</b>	<b>0.11</b>	<b>100</b>
	ST	967	484	217	125	176	106	11	6	17	1554	106	160	3929
	ST %	<b>24.61</b>	<b>12.32</b>	<b>5.52</b>	<b>3.18</b>	<b>4.48</b>	<b>2.70</b>	<b>0.28</b>	<b>0.15</b>	<b>0.43</b>	<b>39.55</b>	<b>2.70</b>	<b>4.07</b>	<b>100</b>
<b>TOTAL</b>		2039 <b>30.38%</b>	1539 <b>22.93%</b>	395 <b>5.88%</b>	303 <b>4.51%</b>	286 <b>4.26%</b>	235 <b>3.50%</b>	42 <b>0.63%</b>	30 <b>0.45%</b>	19 <b>0.28%</b>	1555 <b>23.17%</b>	106 <b>1.58%</b>	163 <b>2.43%</b>	<b>6712</b>

LT: Light trap, ST: Sticky trap

in Turkey, the members of *P. major* group are probable vectors of *L. infantum* (27,40). *Phlebotomus major s.l.* was found to be the dominant species in the study area with a rate of 30.38% (n=2039) in our study. Although this species is found in all villages, 52% of the specimens were collected from Başçayır village (340 m). In the studies conducted in Aydın province, Davies (41) found *P. neglectus* as the fourth dominant species with a rate of 12.66% using LTs while Özbel et al. (18) reported it as the second dominant species with a rate of 26.6% by STs. In our study, two peaks of this species recorded in June and September showed similarity with the results obtained in the study conducted in Çukurova plain, Adana province located in Eastern Mediterranean (33). In another study performed in the Greater region of Athens in 1992 *P. neglectus* had two peaks in June and October (42). While other species caught in the same

area were showing a sharp decrease, *P. neglectus* had a peak in October which can be evaluated as an unusual finding for this geographical region.

*P. tobbi*, a confirmed vector of *L. infantum* in the world, has been reported from the countries in the Eastern Mediterranean (20), Eastern Europe, (43) and the Middle East (44). It was found from all geographical regions in Turkey, and in the studies conducted in the Adana province (Çukurova plain), it was detected as proven vector of *L. infantum* causing cutaneous leishmaniasis CL in this endemic area (31). In other two studies also conducted in Adana province (Çukurova plain), *P. tobbi* was found to be dominant with rates of 67.48% (33) and 71.3% (28). In the present study, around 23% (n=1539) of the total specimens collected were *P. tobbi*. Among 1.539 specimens collected by both types of traps, 59% and 24% of them were collected from two villages (Başçayır



**Table 2.** Seasonal activity features of five probable vector species found in the study area

Species	Total collections n (%)	Active period (from/to)	Seasonal peaks	Month of highest density (%)	Female/male ratio	Altitudinal range m (trap type)
<i>P. major s.l.</i>	2039 (30.38)	April-November	2 peaks June & September	September (32.27)	0.53	200-400 (LT&ST)
<i>P. tobbi</i>	1539 (22.93)	May-October	2 peaks June & September	September (30.28)	1.05	200-400 (LT&ST)
<i>P. papatasi</i>	395 (5.88)	April-October	2 peaks June & end of July	July (19.75)	0.82	200-400 (LT&ST)
<i>P. sergenti s.l.</i>	303 (4.51)	June-October	2 peaks June & end of July	July (18.48)	1.19	600-800 (LT&ST)
<i>P. alexandri</i>	286 (4.26)	May-October	3 peaks June/end of July & September	September (25.52)	3.14	200-400 (LT&ST)

**Table 3.** The percentages of total sand flies caught according to the trap type in different altitudinal range

Altitude (m)	Light trap (%)	Sticky trap (%)	Sticky trap (total specimens/m <sup>2</sup> )	No of species found by both type of traps
0-200	5.23	7.88	8.99	8
<b>200-400</b>	<b>69.71</b>	<b>48.27</b>	<b>55.07</b>	<b>11</b>
400-600	5.41	3.93	4.48	11
600-800	8.17	4.82	5.5	9
800-1000	11.49	35.10	40.04	12

**Table 4.** Species diversity according to the villages at different altitude

Villages	Altitude (m)	No of species	No of specimens	Shannon_H	Shannon_E
<b>Cincin</b>	75	9	243	1.58	0.54
<b>Dağkaraağaç</b>	107	7	178	1.24	0.49
<b>Başçayır</b>	340	10	2314	1.20	0.33
<b>Aşağıkayacık</b>	376	10	816	1.47	0.43
<b>Çarıklar</b>	454	8	119	<b>1.64</b>	0.64
<b>Cumayanı</b>	574	9	114	1.13	0.34
<b>Ortakayacık</b>	655	8	141	<b>1.66</b>	<b>0.66</b>
<b>Akçaköy</b>	780	7	186	1.17	0.46
<b>Karaköy</b>	830	5	60	0.80	0.44
<b>Karatepe</b>	840	12	2541	1.35	0.32

and Aşağıkayacık) located in 200-400 m altitudinal range. In the studies conducted in Aydın province, Davies (41) found *P. tobbi* as dominant species with a rate of 28.66% by LTs while Özbel et al. (18) reported it as the third dominant species with the ratio of 12.1% by STs. In Çukurova plain, sand fly activity starts in the beginning of May and ends in October. *Phlebotomus tobbi* and other sand fly species reach two peaks in August and September, and most of *P. tobbi* specimens were collected in 100-199 m altitudinal range (33). In our study region, we observed two peaks in June and September while the activity period was similar to the previous study (33) that is probably due to similar climate and altitudinal range.

*P. sergenti* and *P. similis* are sister species that are showing allopatric distribution in Old World countries. Significant morphological variation has been reported among *P. sergenti* populations found in Old World countries, and both species have been reported only in Turkey with differences in their distribution areas. *Phlebotomus sergenti* and *P. similis* are previously reported as proven vector species of *L. tropica* in the southeastern and western parts of Turkey, respectively (18,26,45). *Phlebotomus similis* is found in Europe in Greece (46) and Albania (47) and is thought to be a potential vector of ACL. However, in a study conducted with molecular barcoding (*cox1* gene region), it was stated that none of the *P. sergenti* samples examined in western Turkey matched

**Table 5.** Species diversity of caught sand flies according to the collection months

Months	No of species	No of specimens	Shannon	
			H	E
June 2009	11	414	1.88	0.59
July 2009	12	350	<b>2.18</b>	<b>0.74</b>
July 2009	12	874	1.74	0.47
August 2009	10	974	1.65	0.52
August 2009	12	459	1.86	0.53
September 2009	11	1304	1.20	0.30
October 2009	9	304	0.89	0.27
April 2010	2	7	0.59	<b>0.90</b>
May 2010	6	41	1.02	0.46
June 2010	12	973	1.78	0.49
July 2010	11	1009	1.60	0.45

the samples obtained from Crete, therefore, the existence of previously reported *P. similis* species is controversial (36). Since molecular analyzes were not performed in this study, we preferred to report it as *P. sergenti s.l. Phlebotomus sergenti s.l.* was found in all localities in our study, it constituted 4.51% (n=303) of the total species collected from the study area, and it was recorded in Akçaköy (780 m) locality with a maximum abundance of 38% (n=116). While Davies (41) reported this species as *P. similis* with a rate of 5.65%, and Özbel et al. (18) reported it as dominant species (20.33%) and evaluated it as a possible vector of *L. tropica* in the same area. This species showed two peaks in June and the end of July in the study area. In any case, the detection of this species in all localities in the study area was considered as a finding of increased transmission risk of the parasite in these areas.

The anthropophilic species *P. alexandri* showing wide distribution from Spain to China and Africa (7) is a confirmed or probable vector species of *L. donovani* and *L. infantum* in China (48) and Iran (49), respectively. In Turkey, this species was previously notified from almost all VL and CL endemic areas where fauna studies were performed (18), but it is dominant species only in one CL endemic town, Silifke in Mersin Province (50). *Phlebotomus alexandri* remains an important species as a probable vector species of *L. donovani* in Turkey especially after detecting CL and VL cases caused by *L. donovani* in Turkey (51). *Phlebotomus alexandri* was found with the ratio of 1.1% (n=6) by STs in this area previously (18). In the present study, we collected 110 and 176 specimens

(4.26%) by light and STs, respectively. The dominance (n=189) was detected in Başçayır village located in 340 m a.s.l. Most of leishmaniasis vectors detected in the present study showed two peaks in June/July and September while only *P. alexandri* showed three peaks in June, the end of July and September.

The certain vector of ZCL, *P. papatasi* has the widest geographical distribution among Old-World sand fly species (45). It has also wide distribution throughout Turkey (<https://www.kisa.link/PfvY>) with dominance in a highly endemic area of ACL, Şanlıurfa province (26,30). In the present study, we found this species in all altitude ranges with a total of 395 (5.88%) specimens. It showed two peaks in June and the end of July in our study area. The seasonal activity of *P. papatasi* was also investigated in one CL and one VL endemic area. While the Mediterranean climate seen in Çukurova CL endemic areas and has an average altitude of 200-250 m, is similar to our study area, a completely different continental climate is observed in Kars province in a VL endemic area having 2000 m average altitude. It has been determined that the seasonal activity of *P. papatasi* has one or two peaks between June and September in different localities in Çukurova (32,33). On the other hand, due to the short active season of sand flies in Kars province, *P. papatasi* had peaked only in August (52). In a meta-analysis examining 36 studies published between 1994 and 2017 on the seasonal activity of *P. papatasi* conducted in 61 localities in 15 countries in the Old World, it was found that the seasonal activity begins in May and peaks two times between June and September in almost all of these countries. It is stated that three peaks are observed in some localities in the Middle East countries where the active season lasts longer due to the favorable temperature (53). In our study area, it was observed that *P. papatasi* had two peaks in the middle of June and at the end of July. Sand fly fauna studies showed the presence of *P. papatasi* in almost all CL endemic areas of Turkey. For this reason, the distribution and nocturnal/seasonal activity of *P. papatasi* are now more important for Turkey in relation to the increasing autochthonous CL cases caused by *L. major*.

Diversity indices for sand flies have been used by many researchers (28,33,54). Diversity index was also used in our study, and the results showed that although *P. major s.l.* (n=2039) was the dominant species, *P. sergenti s.l.*, a possible vector of CL in the area, was more evenly distributed in Aydın Province than other species. The Shannon-Wiener index (H) indicated no high significant difference between the diversity and abundance of sand fly species distribution in different altitudes (Table 6). Evenness degree and species diversity reached maximum values at 800-1000 m and 0-200 m by light and sticky trap collections, respectively.

*Phlebotomus sergenti s.l.*, *P. major* and *P. tobbi* were found in all localities. Karatepe village (840 m) where all 12 species were

**Table 6.** Species diversity according to the altitudinal range by both types of traps

Altitude (m)	No of species		No of specimens		Shannon_H		Shannon_E	
	LT	ST	LT	ST	LT	ST	LT	ST
	<b>0-200</b>	7	9	147	274	1.29	1.65	0.52
<b>200-400</b>	10	10	1951	1179	1.26	1.40	0.35	0.40
<b>400-600</b>	8	10	149	84	1.35	<b>1.73</b>	0.48	0.56
<b>600-800</b>	8	8	220	107	<b>1.41</b>	1.52	0.51	0.57
<b>800-1000</b>	7	12	316	2285	1.34	1.19	<b>0.54</b>	0.27

LT: Light trap, ST: Sticky trap

found, is in the only locality with both high number of species and specimens. On the other hand, the Shannon index showed the highest species diversity in Ortakayacık (655 m) and Çarıklar (454 m) villages. Regarding sand flies belonging to *Sergentomyia* genus, *S. dentata* was the dominant species, and according to the Shannon index, the species diversity was observed in *S. minuta* the most according to the villages.

## CONCLUSION

In our study, nine and three sand fly species belonging to the *Phlebotomus* and *Sergentomyia* genera were identified, respectively. *Phlebotomus tobbi*, *P. major s.l.* and *P. sergenti s.l.*, which are possible or proven vectors of *L. infantum* and *L. tropica* have been found in the Aydın Mountains and its environs and contribution to the previous fauna studies has been made. It was found that these three dominant species were active for seven months between April and October, *P. sergenti s.l.* was intense in the altitudinal range of 400-600 m in July while *P. major s.l.* and *P. tobbi* were intense in the altitudinal range of 200-400 m in September. These results are important in terms of revealing the time intervals in which the infection in the region is more, months for intense vector control efforts and appropriate vector control strategy to applied.

The results of this study and previous studies show that the activity of sand flies starts in April in Turkey, especially in leishmaniasis endemic areas where the Mediterranean climate is dominant and ends at the end of September after the populations of different species reach one or two peaks depending on the temperature. In the endemic areas located in high altitudes where the continental climate is dominant, it has been determined by studies that the populations can reach only one peak. However, it should be noted that the climate may change every year, and the possibility of seasonal activity of sand flies being slightly different should not be ignored. In addition, it should not be forgotten that the risk of transmission of the parasite may be high when populations show peak(s).

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## \* Ethics

**Ethics Committee Approval:** Not applicable. Our work involved sand flies from the wild.

**Informed Consent:** No patients or animals were involved in the study.

**Peer-review:** Internally peer-reviewed.

## \* Authorship Contributions

Concept: S.K.A., Y.Ö., A.M., Design: S.K.A., Y.Ö., A.M., Data Collection or Processing: S.K.A., Analysis or Interpretation: S.K.A., Y.Ö., A.M., Literature Search: S.K.A., Y.Ö., Writing: S.K.A., Y.Ö.

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