MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF SANDFLY SPECIES (DIPTERA: PSYCHODIDAE) AND THE BIO-ECOLOGY OF CUTANEOUS LESHEIMANIASIS VECTORS IN ERBIL PROVINCE, IRAQ

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Abstract. Phlebotomine sandflies are an important group of insects from a medical perspective that are responsible for the transmission of leishmaniasis. The phenotypic and genotypic investigation of species of sand flies (Al-Harms) as the vector of cutaneous leishmaniasis was conducted in the Erbil province-Iraq for the first time between January and December 2022. Out of 2054 collected samples 1137 (55.4%) were male and 917 (44.6%) were female. statistically, there is no correlation between gender and sandfly species at (P<0.20). Three species were identified as belonging to the genus Phlepotomus: *P. papatasi, P. sergenti*, and *P. Alexandria. P. papatasi* was the most common species, with 1163 (15.6%), followed by *P. sergenti* (598 (29.1%), and *P. alexandri* 293(14.1%). There were two peaks of seasonal abundance of sand flies, the first peak of the seasonally abundant cutaneous leishmaniasis vectors occurred in May, while the second peak occurred in September, statistically, there is a correlation between the periods and sand fly species at (p<0.00). The city's health system must take the necessary precautions to control sand fly species as the vectors of cutaneous leishmaniasis disease. Controlling the sandflies will be a key to controlling (CL) disease.
Keywords: *sandfly, Psychodidae, leishmaniasis, CL, Erbil-Iraq*

Introduction

Phlebotomine sandflies (Diptera: Psychodidae) are proven vectors responsible for the transmission of different species of Leishmania, Leishmania species, the causative agents of leishmaniasis, are known to be transmitted by phlebotomine sandflies (Diptera: Psychodidae) (Moncaz et al., 2012; da Silva Chagas et al., 2018). Sand flies, which comprise roughly 800 species and fall into five main genera (Lutzomyia, Brumptomyia,

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Warileya, and Phlebotomus in the New World, and Sergentomyia, Lutzomyia, and Sergentomyia in the Old World), are the only known carriers of leishmaniasis. Leishmania is only transmitted by species from the genera Phlebotomus and Lutzomyia, though (Bates, 2007).

Leishmaniasis is a complex disease caused by protozoan intracellular parasites, belonging to the genus Leishmania, order Kinetoplastida, family Trypanosomatidae (Garrido-Jareno et al., 2020). There are 4 main forms of the disease: visceral leishmaniasis (VL, also known as kala-azar), post-kala-azar dermal leishmaniasis (PKDL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis. While CL is the most common form of the disease, VL is the most serious and is almost always fatal if untreated (WHO, 2021). The parasites causing leishmaniasis diseases may live and multiply in humans, domestic or stray dogs, and rodents. Multiple strains of the parasite exist, each of which may result in different disease manifestations.

There are 12-15 million cases of leishmaniasis around the world, one to two million new cases are recorded annually. Visceral leishmaniasis is estimated in 500 thousand new cases annually and that 70 thousand of these injuries lead to death development of the disease can take months to years and infected macrophages disseminate through the reticuloendothelial system. Fever, weight loss, anorexia, pallor, diarrhea, epistaxis, hepatosplenomegaly and lymphadenopathy are the 3 common symptoms of leishmaniasis disease (Iddawela et al., 2018). Prevention of leishmaniasis requires a binding of interventional strategies because transmission occurrence in a complex biological mode including the human or animal hosts, parasites, and sandflies vectors (WHO, 2020). WHO postulated key strategies for the prevention of leishmaniasis, which are initial detection and prompt, effective management to reduce the leishmaniasis prevalence and prevent dysfunction and death, vector control to decrease the number of sandflies by using insecticide as a spray or using insecticide–treated nets.

In the developing regions of the world, a sizable category of Neglected Tropical Diseases (NTDs) is known as vector-borne diseases (VBDs). More than a billion individuals are thought to be exposed to these illnesses (Karimian et al., 2018; Valenzuela and Aksoy, 2018). One of the 13 NTDs seen in 88 tropical nations throughout the world is leishmaniasis (Korosh Azizi et al., 2011; Oryan et al., 2013). A variety of Leishmania parasites from the Trypanosomatidae family are the cause of this illness (Davami et al., 2014; Ramezankhani et al., 2017; Parija, 2022). Infectious bites of female phlebotomine sand flies of the Phlebotominae subfamily transmit parasites to humans and other vertebrate hosts (Khosravani et al., 2016; Kourosh Azizi et al., 2016).

“In Iraq, during the late 1940s there was a severe decline in cases of Leishmaniasis, especially in the middle of the 1950s hat decline followed the control of sand flies by insecticides in the malarial eradication scheme and an improvement in general sanitation at the beginning of the 1960s (Guirges, 1971). Poor sanitation, a rise in vector populations and movement of non-immune populations to endemic areas, were the factors behind this surge in leishmaniasis cases. CL Clinical symptoms and serological tests are typically used by doctors to make a diagnosis (WHO, 2014). Nowadays, (sand flies are commonly identified based on morphological features, mostly by internal structures such as the cibarium, pharynx, spermatheca, and terminal genitalia of females and males (Killick-Kendrick, 1995). Knowledge on putative vector species of CL in Iraq is limited and little, species of Phlebotomus like *P. sergenti, P. stoni* and *P. papatasi* are most known in the country.

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Previous Study: Iraqi sand flies were the subject of the earliest published research by Newstead (1920). Sukkar (1974) collected six species of phlebotomine sand flies from six locations within 35 km^2 of Baghdad, similar to Pringle (1953), who had previously conducted a limited survey for sand flies in the Zagros mountains and the central plains of Iraq, then conducted a small-scale survey for sand flies in the Zagros mountains and the central plains of Iraq. In the mountains, only 155 phlebotomine sand flies were found, of which 28% belonged to *P. papatasi* and 28% to *P. sergenti*.

In most of the provinces of Iraq, studies on sand flies have continued. Two phlebotomine species that were identified by morphology are *Phlebotomus sergenti* and *Phlebotomus papatasi*. Between January and December 2016, 1376 male and female sandflies were gathered from five distinct collection regions. The findings indicate that the number of sandflies declined throughout the winter's cold months (December, January, and February) and reached 0%. In contrast, the insect became more active during the warmer months, particularly in August and September, when its percentages were 16.10 and 13.95 percent, respectively (Al-Abbas and associates, 2018).

Oleiwi et al. (2019) carried out a second study in the province of Thi-Qar to look into the species of sand flies that are present there and act as the disease's vector. A total of 6527 sand flies were collected using aspirators, oil traps (sticky papers), and light traps. Of these, 3064 females and 3463 males were distributed. The two species, *P. papaatasi* and *P. sergenti*, belonged to the same genus, Phlepotomus. The most frequent species found were *P. sergenti*, which reached 2056 (31.5%), and *P. papatasi*, which reached 4471 (68.5%). The density of sand flies has two peaks: the first one occurred in May, and the second one occurred in September.

The distribution of the other minor species discovered in this study in Turkey (Toprak and Ozer, 2005). Using morphological and molecular methods, this study was carried out to determine the fauna and yearly activity patterns of sandflies, considering environmental and climatic fluctuations by using morphological tools, focusing on the districts of Erbil province of Iraq, during January-December 2022.

An inquiry was done in Paveh County, Kermanshah Province, west of Iran, to ascertain the biodiversity and seasonal activity of sand flies. Sand flies were caught in five locations in Paveh County between May and October of 2015 using sticky traps. Sand fly activity peaked in early October and ended in late April. 2110 phlebotominae in total (64.6%) were collected outside and 35.4% were collected indoors, with 71.1% of the males and 28.9% of the females. *Phlebotomus alexandri, Ph. Sergenti, Ph. papatasi, Ph. major, Ph. tobbi, Ph. brevis, Sergentomyia sintoni, S. dentata, S. antennata, S. palestinensis, S. pawlowskyi,* and *S. tiberiadis* were among the twelve species in two genera that were identified. In the area, *Phlebotomus alexandri* accounted for 50% of all sand flies collected, while *Ph. brevis* made up just 0.04% of specimens (Mawloudi and associates, 2018).

Materials and Methods

Study area

This research was conducted in the province of Erbil between January 2022 and the end of the same year. The province serves as the administrative center for Iraq's Kurdistan Region (Mojarradgandoukmolla and Akan, 2023). This independent metropolis, which has a total size of 14,873.68 km^2 and is located 350 km north of Baghdad, is the third-largest city in Iraq after Baghdad and Mosul (Kurdistan Regional Government, 2015).

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Seven separate sample sites were selected for the research region, each one reflecting a different environment and human activity across the province's whole urban and rural communities. *Figure 1* illustrates geographic locations where sandflies were collected during this study. Samples were collected by using Aspirators, light traps, and sticky papers collections in the selected areas in Erbil province, Iraq.

Figure 1. *Country (Iraq, Province: Erbil, Districts: Makhmr, Khabat, Gwer, Koya, Soran, Shaqlawa and Center)_ and the geographic locations where sandflies were collected along this study ("The Map Of districts Of Kurdistan Region," 2016)*

Sand flies' collection

Sand flies were gathered between the beginning of January 2022 and the end of December of the same year by using some tools, such as light Traps (L.T), aspirators (ASP) and sticky Paper Traps (S.P) according to the nature of the site in urban and rural zones. (L.T), (ASP) and (S.P) traps were used to collect samples in each sampling area. A total of 2054 sand flies were collected from those regions, 1137 of which were male and 917 of which were female, and all were utilized for morphological identification, population densities, and monthly dispersion. For further confirmation of identification of sand fly species, the molecular identification has been conducted for some collected sand fly samples randomly in the study area. Seventy sand fly collected samples were randomly selected and transferred them into a sterile 1.5 ml micro tube ethanol to molecular identification. Seventy selected individuals were used for genetic species determination. Therefore, a low number of individuals is an ideal situation. If there was genetic diversity analysis, this number would have to be much higher.

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In our study, we used Margalef indices generated by the following as (Topraq, 2005). S-1 /loge N S: Diversity, N is the number of species. The number of individual indices ranges from 0 (least diversity) to 1 (1-1/S). For example, if the research region contains only one species, the diversity is 0 (1-1/S= 0). The simplest similarity measures deal with presence-absence data. According to Sorensen's similarity coefficient we used the below formula (Krebs, 1989). The range of all similarity coefficients for binary data is supposed to be 0 (no similarity) to 1.0 (complete similarity). We also used Temperature records for Erbil and the surrounding areas for 2022 for maximum, lowest, relative humidity, real precipitation by month (Gaznayee et al., 2023 cited in Weather and Climate - The Global Historical Weather and Climate Data) for finding relationships between present or absent of sand flies according to the climate changes in Erbil province.

Morphological identification

Dehydration and dissection

To get rid of castor oil and extra hairs, the sandflies underwent two vigorous washes with distilled water. To avoid stiffing of the sample and drying from alcohol, sand-fly samples were kept in 1.5 mL Eppendorf tubes containing 70% ethanol and drops of glycerin. Labels stating the location and day of the sampling were then fastened. Samples must be preserved for a brief time to avoid deterioration. After that, the samples were put on glass slides. The samples should be properly positioned to enable observation of their morphological and taxonomic traits. On the basis of the sand fly mounted on each plate, external morphological examinations of the several species were conducted. Drops of mounting material (Berleses medium) were used to prevent the creation of air bubbles. The sample was then allowed to dry before the coverslip as gently placed on top of it (Al-Saffar, 2018) performed morphological categorization under a microscope using the keys of the family of sand flies in Iraq, based on internal and exterior morphological features. A light and dissecting microscope were used to perform internal morphological analyses of various organ structures.

Molecular identification

This study used a DNA extraction kit made by Thermo Scientific called the Isolation Kit GeneJET Genomic DNA Purification Kit to molecularly identify sand flies for the first time in the province of Erbil by mitochondrial mtDNA COI and molecular average length 600 base pair of *P. papatasi,* P. *sergenti*, and P. *alexandri*. The mtDNA COI region we chose as the target has the same length and structure at the gene level. However, it varies due to mutations in some parts of different types of nucleotides in this gene region. Due to these differences, species are easily separated from each other in phylogenetic analyses.

DNA extraction

In order to extract DNA from the sand fly samples, individual body ethanol-fixed specimens were homogenized and lysed. Added 200 µl of PBS to a 1.5 ml microcentrifuge tube. Transfered the body of sandfly samples without head and genital into PBS solution, left the specimen at room temperature for 3 hours. At the end of the holding period, added 20 µl of proteinase K to a new tube. Transfer the sandfly samples to this new tube. Add 200 µl of lysis buffer (buffer AL) to it. The samples were Crushed well in this solution. Other steps of the DNA extraction were conducted according to the

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manufacturer of the kite. Finally, the DNA will be ready to put in to the Electrophoresis equipment and then read the bands whether was the DNA extracted or not, the bands will appear or not?

Loading and running DNA in the agarose gel

3 µl DNA was mixed with 3 µl bromophenol blue (loading dye) and loaded into wells of the 0.8-1% agarose gel. The gel was run at 100 V for 30 minutes, and then DNA extracred were examined and visualized by using ultraviolet trans-illuminator (ex: under a UV trans-illuminator) (*Figure 2*).

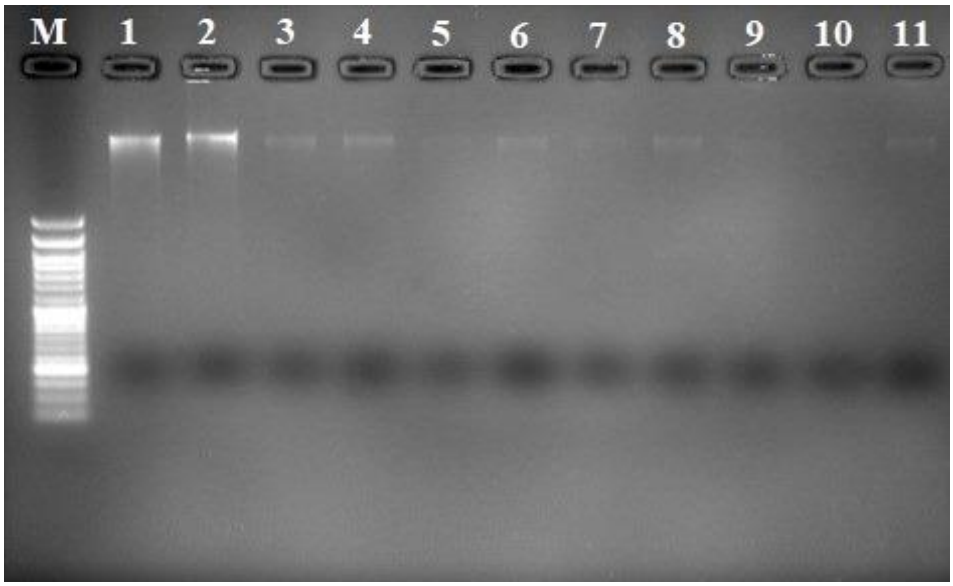

Figure 2. *Total DNA Agarose gel image of some individuals that showed the DNA extracted analysis of mtDNA COI gene in 2% agarose gel at 100 V/cm^2 for 30 min. DNA was visualized under U.V. light after staining with GelStain-GREEN. Where M: DNA ladder, lane (1, 2, 3, 4, 5, 6, 7,8, 9,10 and 11) positive for Phlebotomus species. It is generally considered unnecessary to show all individuals in gel photographs. Therefore, this gel photo was included in our study because it was deemed scientifically appropriate to include one of these gel images as a representative*

PCR amplification of mtDNA COI genes

PCR were used to amplify the genomic DNA and generate an average 600 bp sequence. After adjusting the Polymerase Chain Reaction settings, the 600 bp area of the mtDNA COI was amplified under PCR conditions (*Table 1*). In order to see the bands formed by the oxidized LCO1490 areas as a result of the PCR, a 2% agarose gel was prepared. PCR technique was performed for detection sand flies: *Phlebotomus papatasi*, *Phlebotomus sergenti* and *Phlebotomus alexandri* were designed in this study.

PCR master mix preparation

PCR master mix was prepared by using Maxime PCR Pre Mix and done according to company instructions as following *Table 2*.

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Table 1. *The Conditions of PCR Thermocycler*

PCR cycle	Repeat	Temp.	Time
Initial denaturation	1	95C	3min
Denaturation	40	95C	30sec.
Annealing		48C	30sec
Extension		72C	45sec
Final extension	1	72C	5min

Note: All sand flies species were done at same PCR Thermocycler conditions

Table 2. *Standard PCR reaction for each specimen*

PCR master mix	Volume
Genomic DNA	8µL
Buffer	2.5µL
$MgCl_2$	2µL
Taq DNA polymerase	0.1µL
dNTP	0.5µL
reverse primers (10pmol)	1µL
forward primers (10pmol)	1µL
D.D. water	9.9 µL
Total	25µL

External Thermocycler Reaction Conditions: PCR Thermocycler conditions was done by using (Optimase protocol writer) online application and based on methods described (*Table 1*).

The PCR master mix reaction components were then added to standard PCR tubes containing the PCR PreMix along with the other materials including the components listed in *Table 2* required for the PCR reaction. To mix all the components, the tube was then inserted into a vortex. It was then put into a PCR thermocycler (*Figure 3*).

DNA sequence results

According to a phylogenetic tree analysis using the Phlebotomus species standard NCBI BLAST program, local *Phlebotomus papatasi*, *Phlebotomus sergenti*, and *Phlebotomus alexandri* isolates from various places in the Erbil province were subjected to DNA sequencing. *Phlebotomus papatasi*, *Phlebotomus sergenti*, and *Phlebotomus alexandri* isolates from the local area were used to analyze the mDNA COI gene sequence using M'EGA 6.0, a multiple alignment analysis tool, and the NCBI-Genbank Phlebotomus species based Clustal Walignment analysis. As shown in *Figure 4*, the multiple alignment analysis revealed similarities (*) and differences in the nucleotide sequences of the mDNA COI gene.

The Neighbor Joining (NJ), maximum parsimony, and maximum likelihood approaches were used to infer phylogenetic analyses. The evolutionary distances were calculated using the MEGA 6.0 version of the phylogenetic UPGMA tree type. Phylogenetic trees (*Figure 5*) revealed moderate degrees of interspecific variability between species of the same genus. The full sequencing of the mDNA COI genes of the local *P. papatasi* isolates (Query_367437, Query_367438) demonstrated that they were genetically close to the local *P. papatasi* strains according to NCBI-Blast. The local *P. sergenti* isolates (Query_367439) revealed tight genetic relationships to local *P. sergenti* using NCBI-Blast. The local *P. alexandri* isolates (Query_367435,

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Query_367436) revealed tight genetic relationships to the local *P. alexandri* in NCBI-Blast (*Table 3*). The current work used PCR-direct sequencing to find and identify *P.papatasi* and *P.sergenti* originated from Erbil province endemic CL.

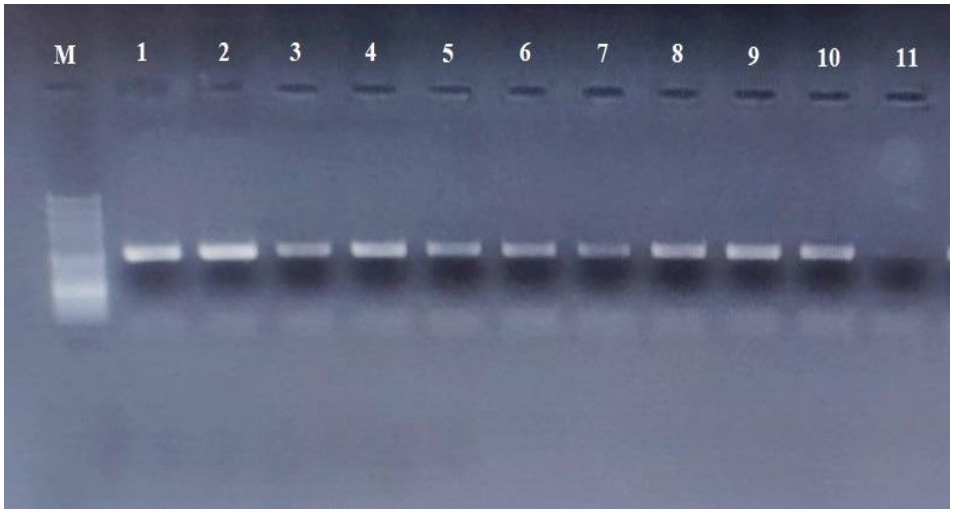

Figure 3. *Image of an agarose gel electrophoresis showing the examination of PCR products from the mDNA COI gene in the gel at 100 V/cm2 for 30 minutes. The PCR product was stained with GelStain-GREEN and then observed under ultraviolet lighting. Where M: DNA ladder; lanes (1, 2, 3, 4,) positive for Phlebotomus papatasi at (600bp); (5, 6, 7) lanes positive for Phlebotomud sergenti at (600bp), (8, 9, 10, and 11) lanes positive for Phlebotomus alexandri at (600bp). After PCR, the marker was used on an agarose gel to measure the length of the products the marker produced. The correct region was found to be oxidized as the target region is 600 bp long. *It is generally considered unnecessary to show all individuals in gel photographs. Therefore, this gel photo was included in our study because it was deemed scientifically appropriate to include one of these gel images as a representative*

Figure 4. *Multiple alignment analysis, multiple sequence alignment performed on the partial mDNA COI gene sequence from local isolates of Phlebotomus papatasi, Phlebotomus sergenti, and Phlebotomus alexandri using M'EGA 6.0, a tool. In the mDNA COI gene nucleotide sequence, the multiple alignment analysis revealed similarities (*) and differences*

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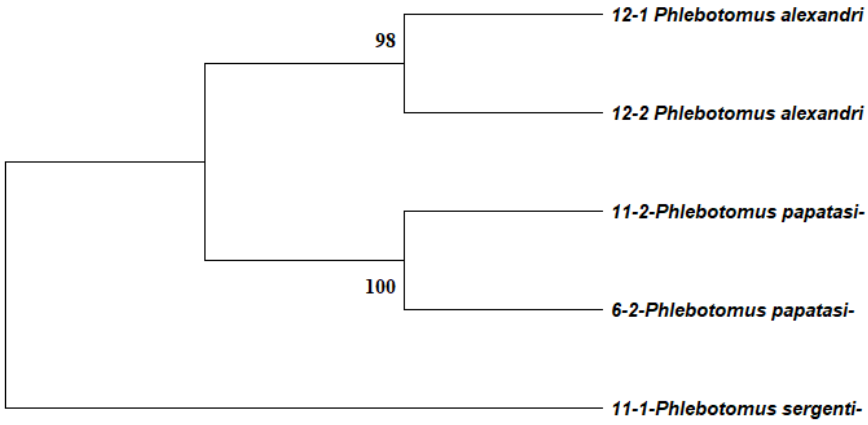
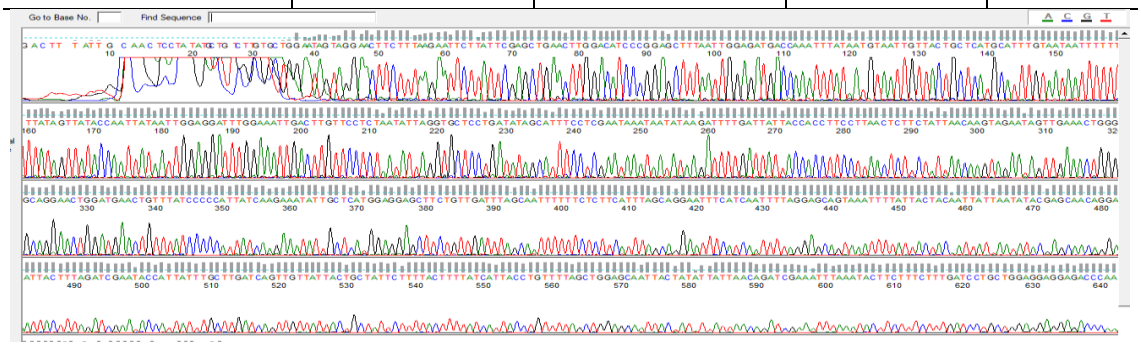


Figure 5. *Neighbor-joining tree of the mDNA COI gene, P. papatasi, P. sergenti, and P. alexandri. The investigation yielded new sequence ancestors for the sandfly species. For each sequence, the sandfly species and GenBank accession numbers are provided*

Table 3. *Homology from NCBI-BLAST Sequence similarities between local isolates of the Phlebotomus papatasi, Phlebotomus sergenti, and Phlebotomus alexandrini species and those in the NCBIGenbank*

Morphological isolate (local isolate)	Genbank submission accession number code	NCBI-BLAST Homology Sequence identity		
		NCBI species name	Accesion number	Identify (100%)
P. papatasi	11-2	*Phlebotomus papatasi*	Query_367437	100
P. papatasi	6-2	*Phlebotomus papatasi*	Query_367438	100
P. alexandri	12-2	*Phlebotomus alexandri*	Query_367436	98
P.alexandri	12-1	*Phlebotomus alexandri*	Query_367435	98
P. sergenti	11-1	*Phlebotomus sergenti*	Query_367439	99



Phylogenetic tree analysis

P. papatasi, *P. sergenti*, and *P. alexandri* were detected and identified in the current investigation using PCR-direct sequencing, which was used to identify endemic CL from the Erbil province. Using the nucleotide sequence, also reveals their phylogenetic link to other identified strains. A polyphyletic species of sand fly, *P. sergenti*, or other insect, *P.*

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papatasi, as well as several other insect species, such as fruit flies, beetles, wasps, and mosquitoes, is present (Kawasaki et al., 2010). Different techniques can be employed in the study of mtDNA findings to establish the genetic separation between species, and Neighbor Joining (NJ) joining diagrams can be created in accordance with these techniques.

Phylogenetic tree

Neighbor-joining tree of the mDNA COI gene, *P. papatasi*, *P. sergenti*, and *P. alexandri*. The investigation yielded new sequence ancestors for the sandfly species. For each sequence, the sandfly species and GenBank accession numbers are provided (*Figure 5*).

Statistical analysis

The data were input into SPSS version 25, and P values of 0.05 or below were considered statistically significant. The chi-square (X2) was used to compare the distribution of discrete independent variables in the current study groups. To describe species abundance trends, several indices of species diversity have been developed, including the Margalef indices and Sorensen's similarity.

Results

Throughout the study, in total, 2054 sand flies were gathered from 7 zones and distributed to 1137 (54.8%) males and 917 (44.2%) females. All specimens were identified morphologically in accordance with the Morphological Classification of Sand Flies key for the Psychodidae family in Iraq (Abul-Hab and Ahmed, 1984). "Three species of the genus Phlebotomus, including *P. papatasi*, *P. sergenti*, and *P. alexandri*, were identified. *P. papatasi* was recorded as the most prevalent sand fly species in the study area, with 1163 (56.6%) individuals, including 663 (58%) males and 500 (43%) females, while, *P.sergenti* a constituted 598 (29.1%) of all sand flies collected, including 321 (53.7%) males, 277 (46.3%) females and *P. alexandri* constituted 293 (14.3%) of all sand flies collected, including 153 (52.2%) males, 140 (47.8%) females. Here is statistically no significant association between gender and sand fly species at (P<0.2)".

Three distinct species of sandflies, all of which are members of the genus Phlebotomus, were identified in the research region, the province of Erbil, between June 2000 and May 2002, utilizing a variety of traps, including light traps, oil paper traps, and aspirators. 2054 sandflies of this species in all, 1137 males and 917 females, were gathered. *Figures 6* and *7* all show the distribution of sandfly species gathered in the sampling areas. This study made clear the variations in sandfly populations throughout a number of province regions (*Figure 1*). Sandflies were most prevalent (32.46%) in the Makhmur region, with a significant difference (P< 0.05).

A total of all specimens was collected. *Phlebotomus papatasi* was the most common species sampled in each habitat. *Phlebotomus papatasi*, constituted (56.6 %) of the total flies collected throughout the study in order of abundance, it was identified in all districts of the province, while *P.sergenti* and *P.alexandri* were not identified in all districts in the province, (*P.sergenti* was identified in all districts except Soran and Shaqlawa districts, while *P.alexandri* was identified only in Makhmur, Khabat and Gwer areas), statistically there are significant differences between sandfly species and districts at (P<0.00). The

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distribution of the species among the sampling stations can be seen in *Figures 7* and *8*. The majority of the sandflies were identified in rural zones reached (75.8%), while the least sandflies were identified in urban zones reached (25.2%), statistically there is no significant difference between the distribution of sandflies and zones (rural and urban) at (P<0.7).

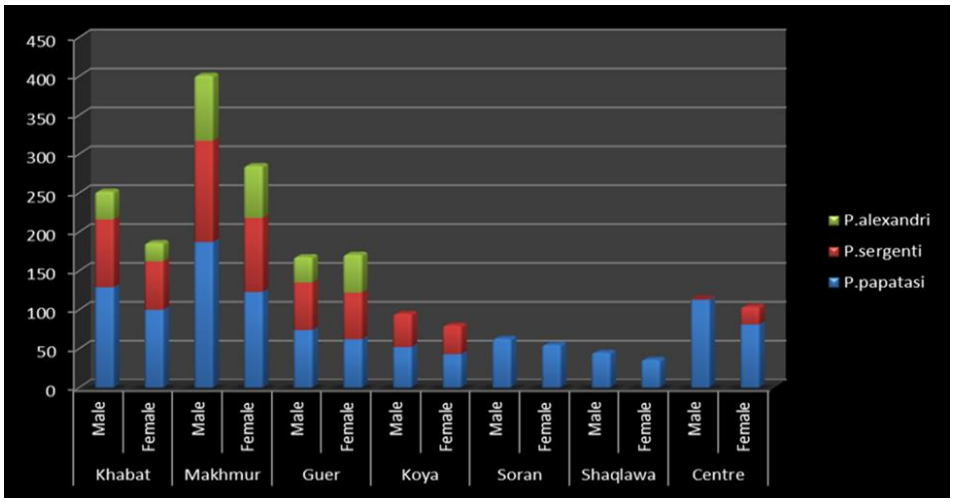

Figure 6. *Distributions of sand fly species in the sampling stations in Erbil province, Iraq, in January-December 2022*

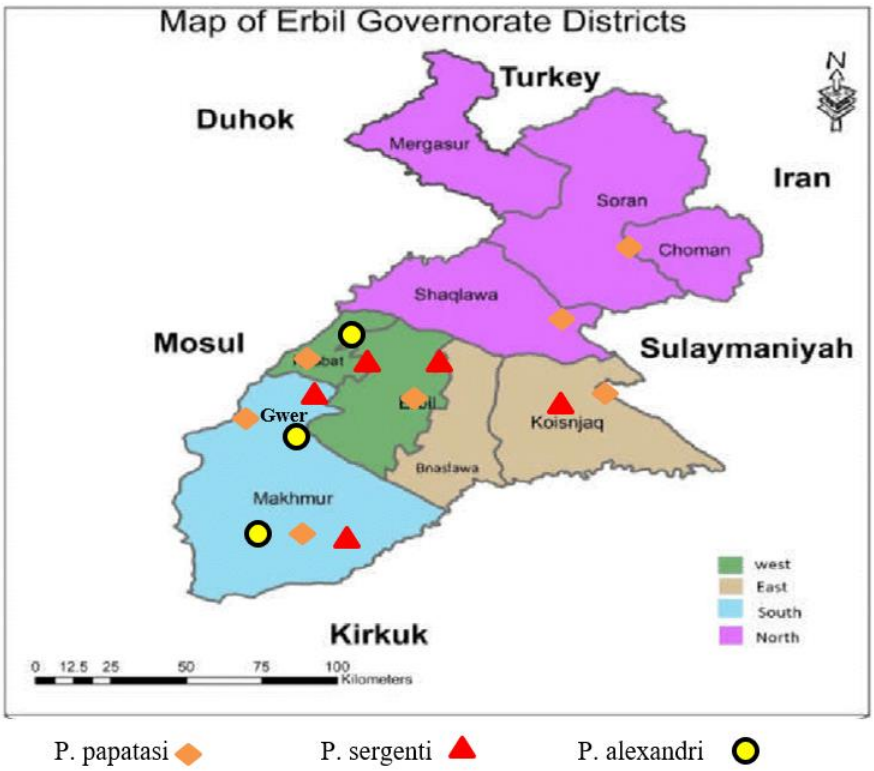

Figure 7. *Map of Distribution of sandfly species belonging to Phlebotomus genera in Erbil province according to sampling stations between January-December 2022*

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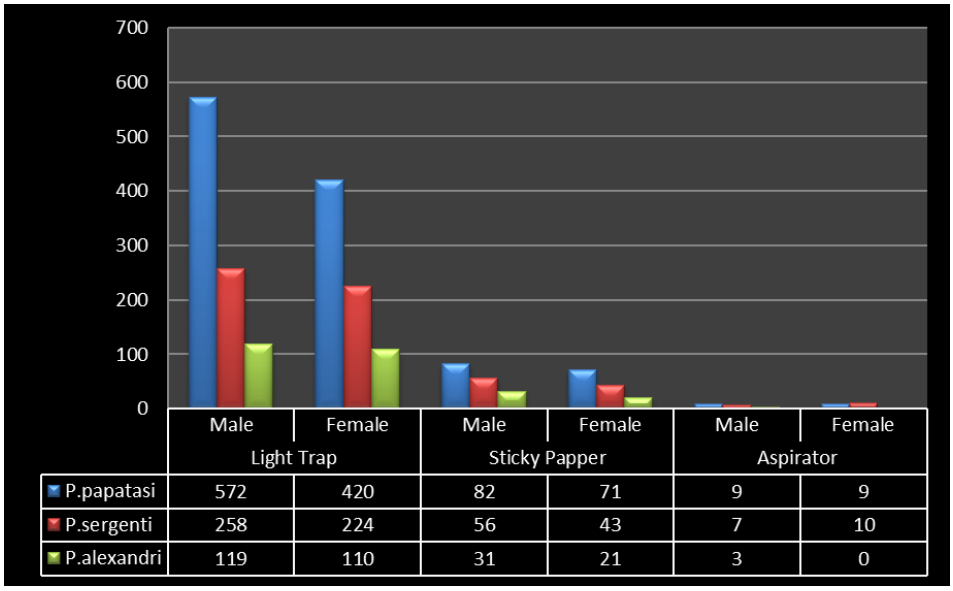

Figure 8. *Trap efficiency to sample for the sandflies species in the study area, Erbil province-Iraq January-December 2022*

The numbers of species, individuals, and biodiversity indices among the districts of the province are shown on *Table 4*. According to these results, Makhmur, Khabat and Gwer districts which are exactly the same in terms of existing species, had the highest biodiversity in terms of sandfly species, but Soran and Shaqlawa districts had no diversity in terms of sand fly species, as diversity equals zero according to the Margalef indices biodiversity equation. However, Erbil center, produced the largest number of sandfly individuals representing a lower number of species. The relevent biodiversity differences for the 3 species of sand fly determined by using Margalef indices biodiversity equation.

Table 4. *Number of the species and individuals of the sandflies captured in the sampling stations of the study area, Erbil province , Iraq in January-December 2022*

Districts	No of the species	No of the individuals	Biodiversity*
Makhmur	3	686	0.70
Khabat	3	438	0.75
Gwer	3	339	0.79
Koya	2	175	0.44
Soran	1	118	0
Shaqlawa	1	81	0
Center	2	217	0.42

* Margalef indices

According to the Sorensons similarity indices, Makhmur, Khabat and Gwer sampling stations have been found to be the most similar stations considering sandfly species. Koya and Erbil City center stations also showed similarity at a rate of 0.8%. But Soran and Shaqlawa sampling stations seemed to be more independent from the other stations and make different clusters (*Table 5*).

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Table 5. *Similarity rates among the sampling stations of sandfly species in Erbil province, January-December 2022*

Sampling station		1	2	3	4	5	6	7
Makhmur	**1**	1	1	1	0.8	0.5	0.5	0.8
Khabat	**2**	1	1	1	0.8	0.5	0.5	0.8
Gwer	**3**	1	1	1	0.8	0.5	0.5	0.8
Koya	**4**	0.8	0.8	0.8	1	0.6	0.6	1
Soran	**5**	0.5	0.5	0.5	0.6	1	1	0.6
Shaqlawa	**6**	0.5	0.5	0.5	0.6	1	1	0.6
Center	**7**	0.8	0.8	0.8	1	0.6	0.6	1

*Soransen's similarity indices

The light trap was most effective for collecting sandfly individuals and all sandfly species. The sticky paper trap came second in order of efficiency and the aspirator trap showed the least efficiency in all collection sites, as seen in *Figure 8*. According to statistical analyses (Pearson Chi-square test), a significant difference was seen among trap preference of the species at $p<0.00$. According to the efficacy of traps in districts, the study reveals that light trap was the most effective trap which captured sandflies in 6 districts, while aspirator trap was the least effective, capturing sandflies only in 3 districts, staticall analysis shows significant associassion between efficiency of traps in districts at $p<0.00$. Out of all sandflies identified, light trap was captured (83.2%) of male sandflies and (83.5%) of female sandflies, but sticky paper captured (15%) male sandflies and (14.2% female sandflies. While aspirator was captured (1.8%) male and (2.3%) female sandflies, there were no significant differences between trap efficiency and the gender at $p<0.2$.

The monthly distribution of three species of sand flies, *P. papatasi*, *P. sergenti* , and *P. alexandri* in Erbil province, is shown in *Figure 4*. *P. papatasi* is observed to begin in February, March, and April to be very rare in June and July, and again observed to increase in August and with high dentistry in May and September but disappearing in January and December. While, *P. sergenti* appeared in March, and April it again reappeared in August and October, with high density in May and September, then disappeared in June, July, November, December, January and February, *P. alexandri* began to appear in March, and April, and it again reappeared in August with high density in May and September, while it disappeared in January, February, June, July, October, November and December. Generally, there were two peaks of existing sandflies along the year, first peak was in May and the second peak was in September. A statistical analysis has shown that there is a significant association between sand fly species of the periods at $p<0.00$ (*Figure 9*).

Sandfly distribution in relation to climate variables during the study's months

This study found a negative correlation between sandfly number and presence, and high temperature, whereas sandflies are active and numerous at moderate temperatures and significantly decline in number during the hot months ($P<0.05$). Sandflies are not affected by rainfall; however, they are more active and abundant in moderate precipitation, and their numbers significantly decline in months with no precipitation (P0. <0.05). Additionally, this study showed that humidity may have an impact on the presence and activity of sandflies ($P<0.05$), sandflies being more active in moderate humidity (*Figure 10* and *Table 6*).

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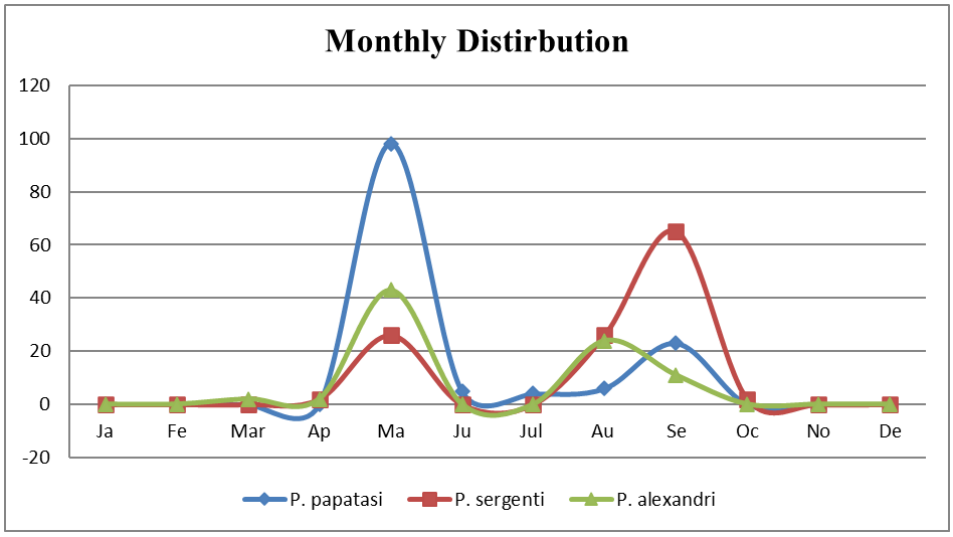

Figure 9. *The monthly distribution of three species of sand flies, P. papatasi, P. sergenti, and P. alexandri in Erbil province January-December 2022*

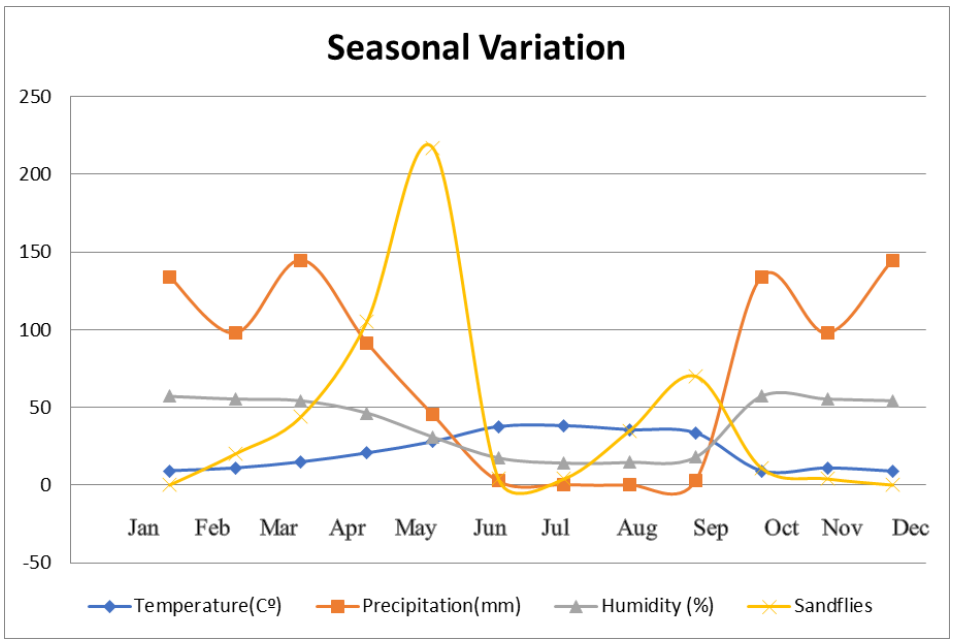

Figure 10. *Seasonal variation of sand fly population in the study's months in Erbil province between January-December2022*

Table 6 shows the seasonal variation of sand fly population in the study's months in Erbil province between January-December 2022.

May, April, and September were the months with the most sandflies observed. where there was a moderate quantity of precipitation, temperature, and humidity. Sandflies can grow and function normally in these circumstances.

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Table 6. *Seasonal variation of sand fly population in the study's months in Erbil province between January-December 2022*

Month	Mean temperature (C°)	Mean Rain amount(mm)	Mean humidity (%)	Sandflies	
				No	(%)
January	9.21	133.9	57.18	0	0
February	10.99	97.7	55.42	79	3.8
March	14.93	94.7	54.34	177	8.6
April	20.69	91.5	46.46	422	20.5
May	27.99	45.4	31.07	867	42.2
June	34.77	2.63	17.56	14	0.7
July	38.35	0.37	14.21	15	0.7
August	37.75	0.19	14.64	139	6.8
September	32.68	3	18.03	284	13.8
October	9.21	133.7	57.18	44	2.1
November	10.99	97.7	55.42	13	0.6
December	14.93	144.7	54.34	0	0
Total / mean±SD	44.33±11.052	23.61±6.511	33.41±11.514		
P-value*	>0.05	>0.05	>0.05		

*Logistic regression test; mm=millimeter; No.=number; %=percentage; SD=standard deviation

Discussion

In the present study, the investigation of sand fly (Diptera:Psycodidae) and the bio-ecology of cutaneous leishmaniasis vectors in Erbil Province of Iraq were done for the first time. The morphological characteristics of *P. papatasi, P. sergenti,* and *P. alexandri* resembled the type key for Diptera and were similar to those described by Abul-Hab and Ahmed (1984): In Iraq, as previously characterized morphologically for both female and male, the Psychodidae family has the ability to transmit certain illnesses, including leishmaniasis due to its abundance and widespread distribution. These findings of the present study were in agreement with a number of earlier studies conducted in Iraq by the pioneers of this field (Sukkar, 1974).

The findings of the present study were consistent with earlier research done in other Iraqi regions by Oleiwi et al. (2019) carried out a second study in the province of Thi-Qar to look into the species of sand flies that are present there and act as the disease's vector. A total of 6527 sand flies were collected using aspirators, oil traps (sticky papers), and light traps. Of these, 3064 females and 3463 males were distributed. The two species, *P. papaatasi* and *P. sergenti*, belonged to the same genus, Phlepotomus. The most frequent species found were *P. sergenti*, which reached 2056 (31.5%), and *P. papatasi*, which reached 4471 (68.5%). The density of sand flies has two peaks: the first one occurred in May, and the second one occurred in September.

Our research was in line with The finding supported the results obtained by Al-Abbas and associates (2018) in An-Najaf province, Iraq, Two phlebotomine species that were identified by morphology are *Phlebotomus sergenti* and *Phlebotomus papatasi*. Between January and December 2016, 1376 male and female sandflies were gathered from five distinct collection regions. The findings indicate that the number of sandflies declined throughout the winter's cold months (December, January, and February) and reached 0%. In contrast, the insect became more active during the warmer months, particularly in August and September, when its percentages were 16.10 and 13.95 percent, respectively.

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The findings of our study are consistent with those of another study that was carried out in the Thi-Qar province by Oleiwi et al. (2019), which looked at the distribution of sand fly species in the region as the vector of cutaneous leishmaniasis. The study collected 6527 sand flies from 15 villages in total, and morphological analysis revealed the existence of two species of the genus Phlebotomus, *P. papatasi* and *P. sergenti*. It has been discovered that *P. papatasi* and *P. sergenti* together now make up the maximum species density. Our study's findings on sandfly monthly distributions were consistent with those of previous research conducted in other countries. Sandfly populations in distinct Panamanian forest habitats were distinguished by significant species variety, geographical heterogeneity, and temporal variation in the study. 48.8% of the collected flies were aspirated from resting locations and caught in light traps near the ground, 12.5% in arboreal light traps, and 38.7% in light traps (Chaniotis et al., 1971). When all sampling techniques were taken into account, light traps were sufficient to identify the local sandfly fauna.

In Turkey Toprak and Ozer (2005) study has not addressed the distribution of the other local minor species. Light traps proved to be the most effective way of capturing all sandfly species that were collected. At all sampling locations, *Phlebotomus papatasi* could be sampled at a rate of 50% using light traps, 35% using aspirators, and 15% using sticky notes. In our investigation, *Phlebotomus papatasi* could be caught using light traps at a rate of 77%, which is consistent with the findings that it is effective. Phlebotumus (93%) and Sergentomyia (7%), as well as four subgenus Phlebotomus (78%), were identified in Syria by Bakdash et al. (2012) in some regions of the Homs province. *P. papatasi* (78%), *P. sergenti* (2%), and other species were recorded for the first time in the study area. *P. papatasi* was the dominant species in the study area.

The findings of our study are consistent with those of another study that carry out by Mawloudi and associates (2018) in Paveh County, Kermanshah Province, west of Iran, to ascertain the biodiversity and seasonal activity of sand flies. Sand flies were caught in five locations in Paveh County between May and October of 2015 using sticky traps. Sand fly activity peaked in early October and ended in late April. 2110 Phlebotominae in total (64.6%) were collected outside and 35.4% were collected indoors, with 71.1% of the males and 28.9% of the females. *Phlebotomus alexandri, Ph. Sergenti, Ph. papatasi, Ph. major, Ph. tobbi, Ph. brevis, Sergentomyia sintoni, S. dentata, S. antennata, S. palestinensis, S. pawlowskyi*, and *S. tiberiadis* were among the twelve species in two genera that were identified. In the area, *Phlebotomus alexandri* accounted for 50% of all sand flies collected, while Ph. brevis made up just 0.04% of specimens.

There were no significant differences between trap efficiency and the gender. The Makmur and Khabat sampling stations had the most sandfly individuals, and they were situated in an area with a lot of agricultural activity. City Center and Koya, which are in the centre of the province and have more urban areas, were found to have more biodiversity than Makhmur and Khabat sample sites, with two sandfly species, but less individuals. In Yuval's study, sandflies were rare in the villages but plentiful in the undisturbed and agriculturally modified environments (Yuval, 1991). Open drainage, organic material rich locations, the animal shelters adjacent to human buildings, and the settlements promote the sandfly habitats in these regions. Looking at similarity indices, Makhmur, Khabat and Gwer sampling stations have been found to be the most similar stations in terms of sandfly fauna. The City center and Koya sampling stations also showed a small similarities at a rate of 0.44 %. But Shaqlawa and Soran sampling stations

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seemed to be different from other stations. These findings were found to be consistent with the different climatic and topographical conditions of the stations.

Our discoveries of *P. papatasi*, *P. sergenti,* and *P. alexandri* in inhabited regions highlight the significance of vector control investigations. *Phlebotomus sergenti* is the proven vector of cutaneous leishmaniasis in the area. It was recorded in the literature that *P. papatasi* is also a vector of the disease. After identifying the entire sandfly fauna of the area and tracking their population dynamics in connection to shifting climatic circumstances and human population migrations, effective control studies should be conducted.

Previous studies about molecular identification of sand flies in Iraq are very rare, except previous studies in Iraq, in Al-Qadisiya province by Al-Hassani (2016) who used method of polymerase chain reaction (PCR) for molecular identification of sand flies for the first time in Iraq through the detection of gene Mitochondrial cytochrome b (Cytb) DNA gene and an output along the molecular 575bp for *P. papatasi* and the length of molecular 325bp for *P. sergenti*. This study used sequencing analysis of the mtDNA COI gene region to verify the results of morphological identification of sandfly species in Erbil province, Iraq, a location with a high prevalence of leishmaniasis sickness. In the genetic examination of populations, nucleotide diversity is a sensitive technique (Nei and Li, 1979). The foundation of biological study is the identification of sand fly species. The external morphology of adults and the features of the cibarium, pharynx, and spermatheca are commonly used to categorize sandflies. However, it can be difficult to identify a species from its physical traits (Zhang et al., 2013).

Conclusion and recommendation

In light of the findings of this study, the percentages of *P. papatasi* were much higher than *P. sergenti* and *P. alexandri* in all districts in Erbil province, it was found in all districts, which illustrated that *P. papatasi* more dangerous than other species in transmitting cutaneous leishmaniasis diseases in the study area. The light trap had the highest success rate (56.6%) at catching sand flies. The first peak of the seasonally abundant cutaneous leishmaniasis vectors occurred in May, while the second peak occurred in early September. There was a considerable statistical difference between the zones (rural and urban sides) and the distribution of sand fly species at ($p<0.00$), with the number of species in rural areas being 1556 (75.8%) and in urban areas being 498 (24.2%). Outcomes of These results can serve as the foundation for the implementation of vector control measures, which may aid in reducing vector density and, as a result, managing cutaneous leishmaniasis in the research region associated with controlling cutaneous leishmaniasis vectors. Public health officials should take advantage of this knowledge to develop optimum vector control tactics in Erbil province and the surrounding area since sand flies are strongly connected with temperature. To further lower the prevalence of cutaneous leishmaniasis in the tested region, effective educational initiatives centered on the disease's transmission and prevention strategies are required, as well as active monitoring to swiftly identify and treat cases.

Despite the fact that this study examined the physical traits and molecular identification of sandfly samples taken from the Erbil province of Iraq, certain conclusions require more evidence for further confirmation because there are more sand fly species in the region. More samples need to be collected for molecular studies in order to confirm the species of sandflies distributed in Erbil province and whether they are

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species complex or not in the future. This is due to the inconsistent nature of some earlier reports and the lack of quantitative observation in this study's molecular identification.

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Conflict of interests. The author has declared that there is no conflict of interests regarding the publication of this article.

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