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Phylogenetic analysis of sandflies populations using cytochrome b (*mtCytb*) gene and identification of Leishmania DNA within infected Sandflies, from the city of Najaf, Iraq

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Objectives Sandflies are the major public health concern in various parts of the world. The aim of this study is to identify the species and strain of sandflies, using molecular methods.
Methods Sandflies were collected from January to October 2017, in 16 rural areas in the province of Najaf AL-Ashraf, Iraq. Polymerase chain reaction technique was performed for detection of mitochondrial cytochrome b (*mtCytb*) gene in *Phlebotomus papatasi (P. papatasi)*, *Phlebotomus sergenti (P. sergenti)*, and *Sergentomyia sintoni (S. sintoni)*. DNA sequencing method was performed for confirmatory identification of *P. papatasi, P. sergenti* and *S. sintoni* from local isolates based on *mtCytb*, using phylogenetic tree analysis (MEGA.6) and NCBI-BLAST multiple sequence alignment tool.
Results Morphological identification of sandflies shows that all specimens were categorized into two genera with three species, *Phlebotomus* and *Sergentomyia*. *Leishmania* DNA was detected in 16 pools, all were infected with *Leishmania major*, eight of them infected with *Leishmania tropica*. Sequencing and phylogenetic inference analysis confirmed that the local *P. papatasi* isolates were demonstrated to be closely related to the NCBI, *P. papatasi* reference sequence (AF161214.1), the local *P. sergenti* isolates showed high similarity with the NCBI, *P. sergenti* sequence (AF161216.1), and the local *S. sintoni* isolates showed high homology with the NCBI, *S. sintoni* sequence (EU159507.1).
Conclusions *P. papatasi, P. sergenti* and *S. sintoni* were the genotypes that has a high prevalence in the city of Najaf. No previous data were found in this regard. The present study contributes to a better understanding of the molecular epidemiology of this parasite.
Keywords *leishmania*, vector, sequence

Introduction

Sandflies are the major public health problem, worldwide. The Middle East region, including Iraq, is highly endemic for *Phlebotomine* sandfly vector and so, for leishmaniasis. Approximately, 98 out of 800 described sandfly *spp*. are suspected vectors of human leishmaniasis, among them 42 are *Phlebotomus* species found in the old world (Sharma et al. 2017).

The lack of a human vaccine to the available drugs and their serious side effects urge the scientists to further study and focus not only on the parasite itself, but also its hosts and vectors. Considering the resurgence of leishmaniasis in some non-endemic areas of Iraq, scientists have been attracted to cutaneous leishmaniasis, but as molecular data on sandflies are limited, these studies have become the basis for novel approaches to reduce transmission of several insect-borne diseases.

Some studies indicated that the identification of vector is important for implementing the controlling strategy, in other studies, the researchers stated that the vector-targeted studies are necessary from the time when the vector has the ability to transmit infectious diseases to humans.[1]

This study aims to identify the sandflies, using polymerase chain reaction (PCR) amplification and sequencing analysis to determine the possible vectors in study areas.

Materials and Methods

Study areas

Sandflies were collected from January to October 2017, from 16 rural areas in the province of Najaf AL Ashraf, by focusing on the cutaneous leishmaniasis. Large numbers of cutaneous leishmaniasis cases were reported from 2003 to 2016. Najaf Al Ashraf is a city in central Iraq, about 160 km (100 miles) south of Baghdad. It is the capital of Nafaf.

Sandflies collection

Sandflies collection was performed, using manual aspirators and torches from their resting sites (inside houses of affected individuals as notified to the local health directorate), on the ceilings and wall of bedrooms and bathrooms of houses, during the early morning hours. Centers for Disease Control (CDC) light traps, located about 1.5 m above the land, were set before sunset and collected the next morning, inside houses at the study sites.

Processing and storage of collected sandflies

The collected sandflies placed into a cooler box, with wet paper towels lining and ice packs, then placed into the freezer (−20°C) for a few minutes. Typically, collected sandflies included more than one species and many other insect genera. The specimens that used for the taxonomic purpose were preserved dry, in layers of tissue paper, prior to being cleared in chloroform. The specimens were stored in secure vials or tubes filled to the top with 95% ethanol (for PCR applications) and were bearded stable labels, identifying the collection, place and time.

Morphological identification of sandflies

The morphological identification was determined based on the characteristics of the head, abdominal Terminalia and coxite hairs, using compound microscopy (400×).

DNA extraction

DNA was extracted from female sandflies, using the Tissue Genomic DNA mini kit, from Geneaid Biotech Ltd. (Taiwan) and completed the steps, based on the Company's guidelines. Extracted DNA was kept at −20°C, until PCR was performed.

Polymerase chain reaction

Polymerase chain reaction technique was performed for detection of *mtCytb* in *P. papatasi*, *P. sergenti*, and *S. sintoni*. This technique was performed, according to the method described by Raja et al.[2] Specific primers were designed in this work from highly conserved regions of *mtCytb* and supplied by the Bioneer Company.

The PCR master mix was prepared, according to the kit instructions as follows (Table 2).

These components were added to the premix pellet in a premix tube, then were mixed by a vortex. The PCR thermocycler conditions were as follows: An initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 60°C for 1 min and 72°C for 1 min, and a final elongation at 72°C for 5 min.

The final PCR products were subjected to electrophoresis on a 1% agarose gel with ethidium bromide stain, and visualized under UV transilluminator.

DNA sequencing method

DNA sequencing was performed for confirmatory detection of *P. papatasi*, *P. sergenti* and *S. sintoni* in local isolates based on *mtCytb*, using phylogenetic tree analysis (MEGA.6) and NCBI-BLAST, multiple sequence alignment tool. The PCR product was purified from the agarose gel, using the EZ-10 Spin Column DNA Gel Extraction Kit (Bio Basic, Canada). The DNA sequencing, using forward primer (AB DNA sequencing system) was performed by Macrogen Company in Korea.

Table 1. **The primer sequences used for PCR amplification**

Primer		Sequence (5′-3′)	Amplicon
P. papatasi	F	TCCGCCATCCCTTATCTAGG	575 bp
	R	GGACGAGCTCCGATTCATGT	
P. sergenti	F	GTCCAATGAATCTGAGGAGGGT	325 bp
	R	GAATGTGGGGAGGGGTTACT	
S. sintoni	F	TGAGGAGGATTCGCCGTAGA	575 bp
	R	ACGGTTAAAATTTGACCTGTGAGA	
Leishmania spp.	F	ACTGGGGGTTGGTGTAAAATAG	560 pb L. *major*
	R	TCGCAGAACGCCCCT	750 bp L. *tropica*

Table 2. **The PCR master mix**

PCR master mix		Volume
Genomic DNA		5 µl
primers	F	1µl of 10 pmol
	R	1µl of 10 pmol
PCR water		13µl
Total		20 µl

Results

Morphological identification of sandflies

All specimens were identified morphologically, according to the criteria published by Jalil Abul-hab (1984), Al-Dawood et al. (2004) and categorized into two genera, *Phlebotomus* and *Sergentomyia*. *P. papatasi* was identified from all the 16 pools (100%), *P. sergenti* was found in 8 (50%) of the 16 study areas, while the *S. Sintoni* represented 25% of the samples in four pools. Thus *P. papatasi* was the predominant members in collected sandflies at all locations of this study areas.

Leishmania DNA detection

Leishmania DNA was detected in 16 pools. In total, 16 pools (100%) were infected by *Leishmania spp.*, all 16 pools were infected with *Leishmania major*, among them eight were infected with *Leishmania tropica*, considering that at least one specimen was infected in each positive pool.

Sandflies DNA detection

The study revealed that 16 pools (100%) were positive after PCR amplification, while none of the specimens were negative for the parasite. *P. papatasi* was detected in all 16 pools, eight pools were positive for *P. sergenti*, but only four pools were positive for *S. sintoni*.

Sequencing and phylogenetic inference analysis

The sandflies sequence deposited in GenBank and the *mtCytb* that used for confirmatory identification were aligned, using the Unweighted Pair Group method and by calculating the Arithmetic Mean (UPGMA tree), usingMEGA 6.0 version. The phylogenetic tree analysis show that the local *P. papatasi* isolates were closely related to the *P. papatasi* reference sequence available in the NCBI (AF161214.1), the local *P. sergenti* isolates were similar to *P. sergenti* sequence (AF161216.1), and the local *S. sintoni* isolates showed high homology with the NCBI, *S. sintoni* sequence (EU159507.1) (Fig. 4).

Discussion

The sandflies, the vectors of leishmaniasis have received considerable attention in recent years, in different parts of the world, due to the recovery of leishmaniasis in some non-endemic areas.[3]

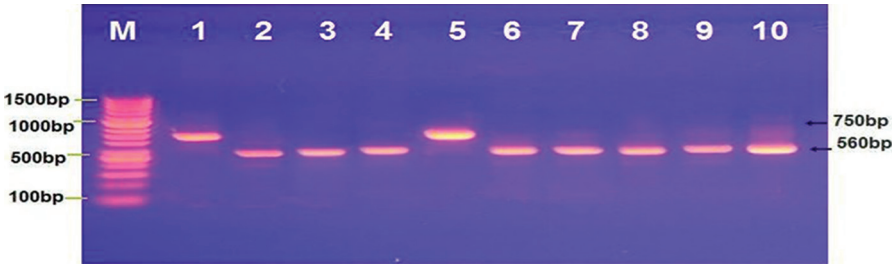

Fig. 1 **Agarose gel electrophoresis of *Leishmania* isolates, where M: marker (100–1500 bp), lane 1 and 5 (750 pb) *L. tropica isolates* , Lanes 2–4, 6, 7–10 (560pb) *L. major* isolates.**

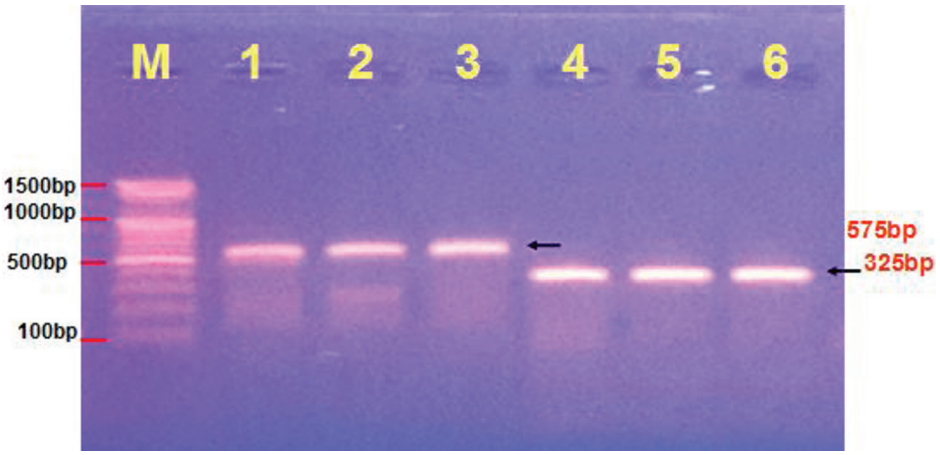

Fig. 2 **Agarose gel electrophoresis of *Phlebotomus spp*. isolates after PCR amplification. Lane M, DNA size marker (100–1500 bp), lanes 1–3 (575 bp) *P. papatasi* isolates; lanes 4–6 (325 bp) *P. sergenti*.**

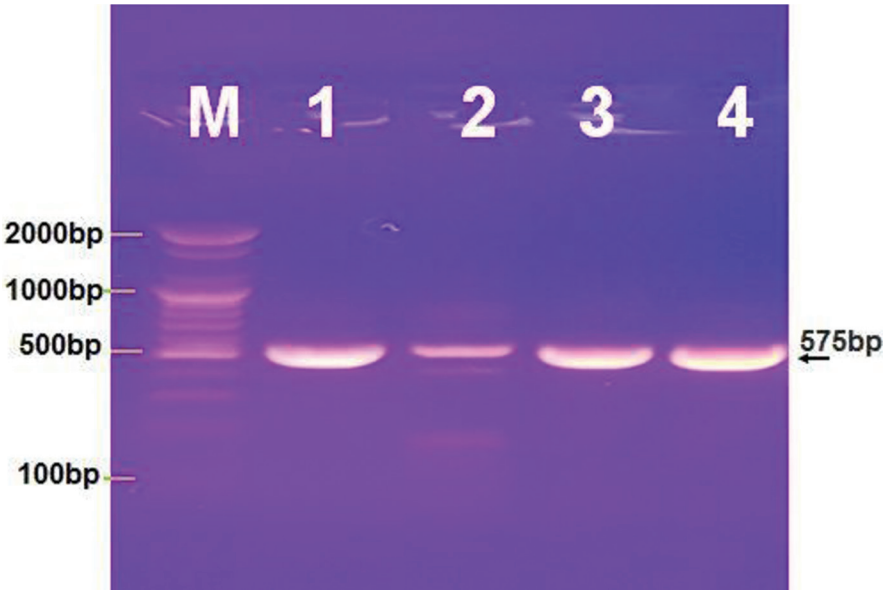

Fig. 3 **Agarose gel electrophoresis of *Sergentomyia* spp. isolates in PCR. Lane M, DNA size marker (100–2000 bp), lanes 1–4 (575 bp) *S. sintoni* isolates.**

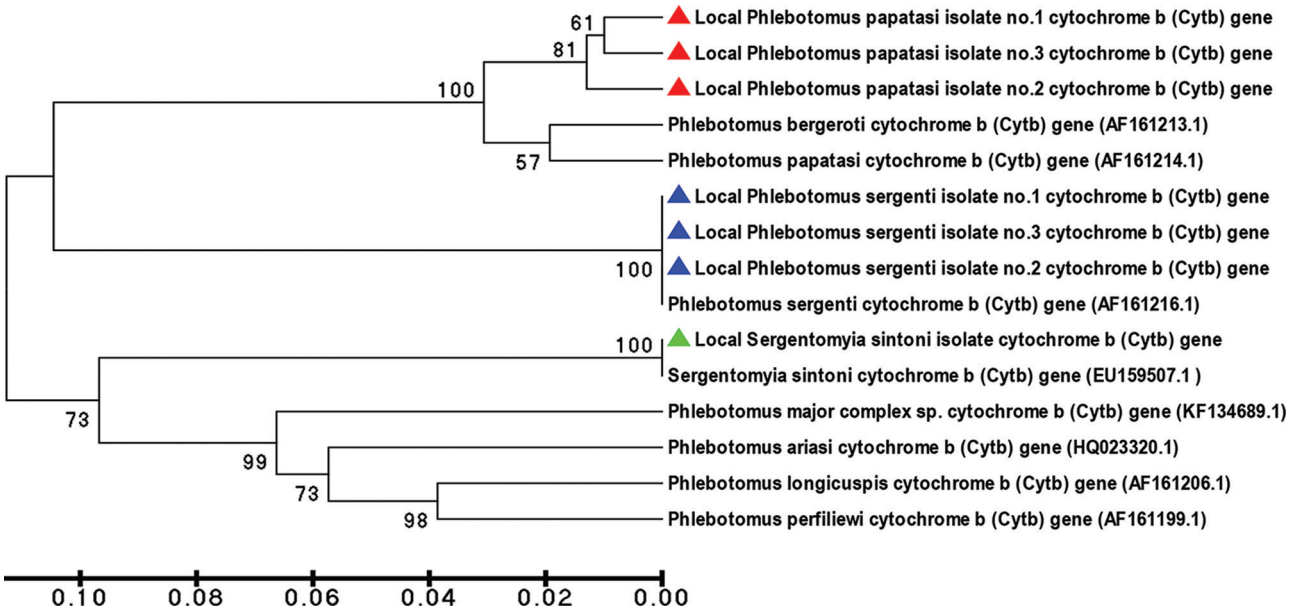

Fig. 4 **Phylogenetic tree analysis show that the local *P. papatasi* isolates (1–3) were closely related to the NCBI *P. papatasi* sequence (AF161214.1), the local *P. sergenti* isolates (1–3) were similar to the NCBI, *P. sergenti* sequence (AF161216.1), and the local *S. sintoni* isolates showed high homology with the NCBI, *S. sintoni* sequence (EU159507.1).**

Table 3. **The confirmatory identification of local *P. papatasi* and local *P. sergenti*, using mtCytb partial sequence, according to phylogenetic tree analysis and NCBI-BLAST alignment tool**

Isolate no.	NCBI-BLAST homology sequence identity (%)		Amplicon
	Phlebotomus papatasi (AF161214.1) (%)	*Phlebotomus sergenti* (AF161216.1) (%)	*Sergentomyia sintoni* (EU159507.1) (%)
Local *P. papatasi* isolate no. 1	99	-	-
Local *P. papatasi* isolate no. 2	99	-	-
Local *P. papatasi* isolate no. 3	99	-	-
Local *P. sergenti* isolate no. 1	-	100	
Local *P. sergenti* isolate no. 2	-	100	-
Local *P. sergenti* isolate no. 3	-	100	
Local *S. sintoni* isolate	-	-	100

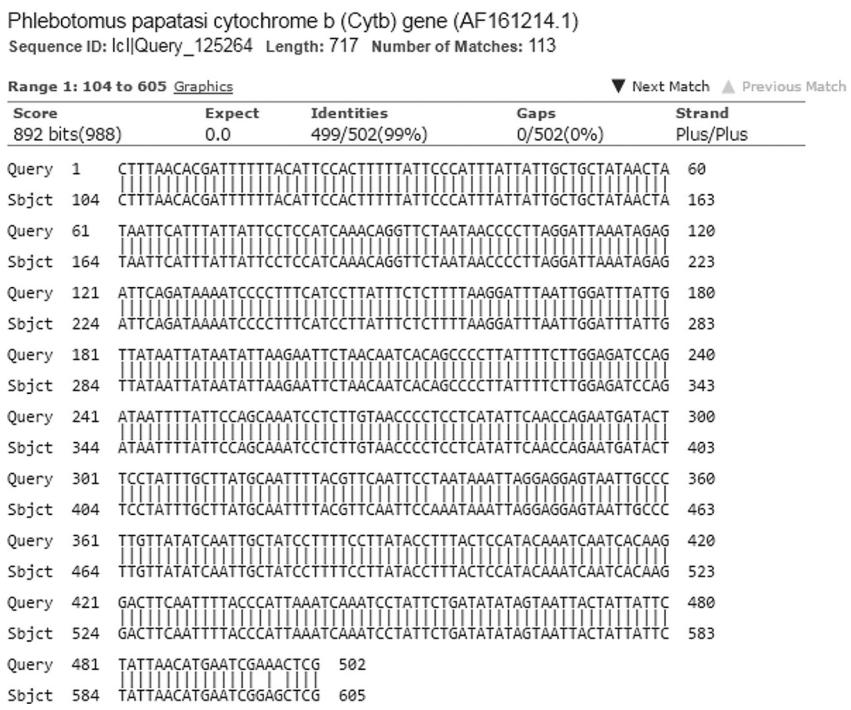

Fig. 5 **Pairwise sequence alignment of the *Cytb of P. papatasi* isolate no. 1 with the NCBI *P. papatasi* sequence (AF161214.1), showing 99% homology, 499 bp out of 502 bp.**

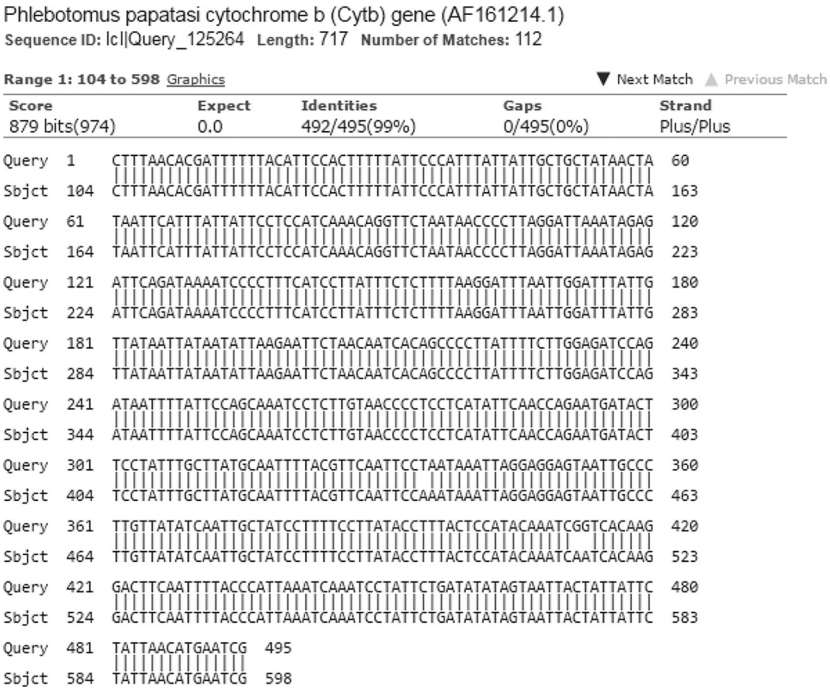

Fig. 6 **Pairwise sequence alignment of the *Cytb of P. papatasi* isolate no. 2 with the NCBI *P. papatasi* (AF161214.1), showing 99% identity, 492 bp out of 495 bp.**

Phlebotomus papatasi cytochrome b (Cytb) gene (AF161214.1)
Sequence ID: lcl|Query_125264 **Length:** 717 **Number of Matches:** 113

Range 1: 104 to 605 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
897 bits(994)	0.0	500/502(99%)	0/502(0%)	Plus/Plus

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Query  1    CTTTAACACGATTTTTTACATTCCACTTTTTATTCCCATTTATTATTGCTGCTATAACTA  60
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Sbjct  104  CTTTAACACGATTTTTTACATTCCACTTTTTATTCCCATTTATTATTGCTGCTATAACTA  163

Query  61   TAATTCATTTATTATTCCTCCATCAAACAGGTTCTAATAACCCCTTAGGATTAAATAGAG  120
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Sbjct  164  TAATTCATTTATTATTCCTCCATCAAACAGGTTCTAATAACCCCTTAGGATTAAATAGAG  223

Query  121  ATTCAGATAAAATCCCCTTTCATCCTTATTTCTCTTTTAAGGATTTAATTGGATTTATTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  224  ATTCAGATAAAATCCCCTTTCATCCTTATTTCTCTTTTAAGGATTTAATTGGATTTATTG  283

Query  181  TTATAATTATAATATTAAGAATTCTAACAATCACAGCCCCTTATTTTCTTGGAGATCCAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  284  TTATAATTATAATATTAAGAATTCTAACAATCACAGCCCCTTATTTTCTTGGAGATCCAG  343

Query  241  ATAATTTTATTCCAGCAAATCCTCTTGTAACCCCTCCTCATATTCAACCAGAATGATACT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  344  ATAATTTTATTCCAGCAAATCCTCTTGTAACCCCTCCTCATATTCAACCAGAATGATACT  403

Query  301  TCCTATTTGCTTATGCAATTTTACGTTCAATTCCTAATAAATTAGGAGGAGTAATTGCCC  360
            ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  404  TCCTATTTGCTTATGCAATTTTACGTTCAATTCCAAATAAATTAGGAGGAGTAATTGCCC  463

Query  361  TTGTTATATCAATTGCTATCCTTTTCCTTATACCTTTACTCCATACAAATCAATCACAAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  464  TTGTTATATCAATTGCTATCCTTTTCCTTATACCTTTACTCCATACAAATCAATCACAAG  523

Query  421  GACTTCAATTTTACCCATTAAATCAAATCCTATTCTGATATATAGTAATTACTATTATTC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  524  GACTTCAATTTTACCCATTAAATCAAATCCTATTCTGATATATAGTAATTACTATTATTC  583

Query  481  TATTAACATGAATCGAAGCTCG  502
            ||||||||||||| ||||||
Sbjct  584  TATTAACATGAATCGGAGCTCG  605
```

Fig. 7 **Pairwise sequence alignment of the *Cytb of P. papatasi* isolate no. 3 with *P. papatasi* (AF161214.1), showing 99% identity, 500 bp out of 502 bp.**

Phlebotomus sergenti cytochrome b (Cytb) gene (AF161216.1)
Sequence ID: lcl|Query_125263 **Length:** 717 **Number of Matches:** 72

Range 1: 64 to 394 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
598 bits(662)	2e-175	331/331(100%)	0/331(0%)	Plus/Plus

```
Query  1    GTCCAATGAATCTGAGGAGGGTTTGCTGTTGATAATGCTACTTTAACACGCTTTTTCACC  60
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Sbjct  64   GTCCAATGAATCTGAGGAGGGTTTGCTGTTGATAATGCTACTTTAACACGCTTTTTCACC  123

Query  61   TTTCATTTTTTATTCCCGTTTATTATAGCCGCAATAACAATAATCCATCTATTGTTCCTC  120
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Sbjct  124  TTTCATTTTTTATTCCCGTTTATTATAGCCGCAATAACAATAATCCATCTATTGTTCCTC  183

Query  121  CACCAAACAGGATCAAATAACCCCTTTGGACTAAACAGAAATTCTGACAAAATCCCATTT  180
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Sbjct  184  CACCAAACAGGATCAAATAACCCCTTTGGACTAAACAGAAATTCTGACAAAATCCCATTT  243

Query  181  CATCCTTACTTTTCTTTCAAGGATTTTATTGGATTTATTTTAATAACAATAGCTCTCGTA  240
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Sbjct  244  CATCCTTACTTTTCTTTCAAGGATTTTATTGGATTTATTTTAATAACAATAGCTCTCGTA  303

Query  241  TTTTTAACTATTATTGCCCCCTATTTTTTAGGAGACCCAGATAATTTTATTCCAGCAAAT  300
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Sbjct  304  TTTTTAACTATTATTGCCCCCTATTTTTTAGGAGACCCAGATAATTTTATTCCAGCAAAT  363

Query  301  CCTTTAGTAACCCCTCCCCACATTCAACCAG  331
            |||||||||||||||||||||||||||||||
Sbjct  364  CCTTTAGTAACCCCTCCCCACATTCAACCAG  394
```

Fig. 8 **Pairwise sequence alignment of the *Cytb of P. sergenti* isolate no. 1 with NCBI *P. sergenti* sequence (AF161216.1), showing 100% identity, 331 bp out of 331 bp.**

Phlebotomus sergenti cytochrome b (Cytb) gene (AF161216.1)
Sequence ID: lcl|Query_125263 **Length:** 717 **Number of Matches:** 72

Range 1: 64 to 391 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
592 bits(656)	8e-174	328/328(100%)	0/328(0%)	Plus/Plus

```
Query  1    GTCCAATGAATCTGAGGAGGGTTTGCTGTTGATAATGCTACTTTAACACGCTTTTTCACC  60
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Sbjct  64   GTCCAATGAATCTGAGGAGGGTTTGCTGTTGATAATGCTACTTTAACACGCTTTTTCACC  123

Query  61   TTTCATTTTTTATTCCCGTTTATTATAGCCGCAATAACAATAATCCATCTATTGTTCCTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  124  TTTCATTTTTTATTCCCGTTTATTATAGCCGCAATAACAATAATCCATCTATTGTTCCTC  183

Query  121  CACCAAACAGGATCAAATAACCCCTTTGGACTAAACAGAAATTCTGACAAAATCCCATTT  180
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Sbjct  184  CACCAAACAGGATCAAATAACCCCTTTGGACTAAACAGAAATTCTGACAAAATCCCATTT  243

Query  181  CATCCTTACTTTTCTTTCAAGGATTTTATTGGATTTATTTTAATAACAATAGCTCTCGTA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  244  CATCCTTACTTTTCTTTCAAGGATTTTATTGGATTTATTTTAATAACAATAGCTCTCGTA  303

Query  241  TTTTTAACTATTATTGCCCCCTATTTTTTAGGAGACCCAGATAATTTTATTCCAGCAAAT  300
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Sbjct  304  TTTTTAACTATTATTGCCCCCTATTTTTTAGGAGACCCAGATAATTTTATTCCAGCAAAT  363

Query  301  CCTTTAGTAACCCCTCCCCACATTCAAC  328
            ||||||||||||||||||||||||||||
Sbjct  364  CCTTTAGTAACCCCTCCCCACATTCAAC  391
```

Fig. 9 **Pairwise sequence alignment of the *Cytb of P. sergenti* isolate no. 2 with *P. sergenti* (AF161216.1), showing 100% identity, 328 bp out of 328 bp.**

Phlebotomus sergenti cytochrome b (Cytb) gene (AF161216.1)
Sequence ID: lcl|Query_125263 **Length:** 717 **Number of Matches:** 72

Range 1: 64 to 388 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
587 bits(650)	3e-172	325/325(100%)	0/325(0%)	Plus/Plus

```
Query  1    GTCCAATGAATCTGAGGAGGGTTTGCTGTTGATAATGCTACTTTAACACGCTTTTTCACC  60
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Sbjct  64   GTCCAATGAATCTGAGGAGGGTTTGCTGTTGATAATGCTACTTTAACACGCTTTTTCACC  123

Query  61   TTTCATTTTTTATTCCCGTTTATTATAGCCGCAATAACAATAATCCATCTATTGTTCCTC  120
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Sbjct  124  TTTCATTTTTTATTCCCGTTTATTATAGCCGCAATAACAATAATCCATCTATTGTTCCTC  183

Query  121  CACCAAACAGGATCAAATAACCCCTTTGGACTAAACAGAAATTCTGACAAAATCCCATTT  180
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Sbjct  304  TTTTTAACTATTATTGCCCCCTATTTTTTAGGAGACCCAGATAATTTTATTCCAGCAAAT  363

Query  301  CCTTTAGTAACCCCTCCCCACATTC  325
            |||||||||||||||||||||||||
Sbjct  364  CCTTTAGTAACCCCTCCCCACATTC  388
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Fig. 10 **Pairwise sequence alignment of the *Cytb* of *P. sergenti* isolate no. 3 with the NCBI *P. sergenti* (AF161216.1), showing 100% identity, 325 bp out of 325 bp.**

Sergentomyia sintoni haplotype IRN509 cytochrome b gene, partial cds; mitochondrial
Sequence ID: EU159507.1 **Length:** 708 **Number of Matches:** 1

Range 1: 67 to 614 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1013 bits(548)	0.0	548/548(100%)	0/548(0%)	Plus/Plus

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Query  1    TGAGGAGGATTCGCCGTAGATAATGCAACCTTAACTCGATTTTTTACATTTCATTTCTTA  60
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Query  61   TTCCCTTTTATTGTTGCAGCAATAACAATAATCCACCTATTATTTCTTCATCAAACTGGG  120
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Query  121  TCTAATAACCCTCTAGGACTAAATAGTAATAGAGATAAAATTCCTTTTCATCCTTATTTT  180
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Sbjct  187  TCTAATAACCCTCTAGGACTAAATAGTAATAGAGATAAAATTCCTTTTCATCCTTATTTT  246

Query  181  TCATTTAAAGATTTAATTGGATTTATTATCATATTAATACTTCTAACCTTCTTAACAATT  240
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Query  241  ATTAGTCCATATTTTTTAGGAGATCCTGATAATTTTATTCCAGCTAATCCCTTAGTTACA  300
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Sbjct  307  ATTAGTCCATATTTTTTAGGAGATCCTGATAATTTTATTCCAGCTAATCCCTTAGTTACA  366

Query  301  CCCCCTCATATTCAGCCTGAATGATATTTTCTGTTTGCTTATGCAATTCTTCGTTCAATT  360
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Sbjct  367  CCCCCTCATATTCAGCCTGAATGATATTTTCTGTTTGCTTATGCAATTCTTCGTTCAATT  426

Query  361  CCTAATAAATTAGGTGGAGTTATTGCACTAGTTATATCTATTGCAATTTTATTTGTCCTT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  427  CCTAATAAATTAGGTGGAGTTATTGCACTAGTTATATCTATTGCAATTTTATTTGTCCTT  486

Query  421  CCAATTCTTCATGTTAGTAAATCCCAAGGTTTACAATTTTATCCAATTAACCAAATTCTT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  487  CCAATTCTTCATGTTAGTAAATCCCAAGGTTTACAATTTTATCCAATTAACCAAATTCTT  546

Query  481  TTTTGATATATAGTTATTATTATTGTTCTATTAACTTGAATTGGAGCCCGCCCAGTTGAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  547  TTTTGATATATAGTTATTATTATTGTTCTATTAACTTGAATTGGAGCCCGCCCAGTTGAA  606

Query  541  GACCCTTA  548
            ||||||||
Sbjct  607  GACCCTTA  614
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Fig. 11 **Pairwise sequence alignment of the *Cytb* of *S. sintoni* isolate, with the NCBI *S. sintoni* (EU159507.1), showing 100% identity, 325 bp out of 325 bp.**

Although, data on the sandflies population of Iraq was accumulated, this is the first study of the species composition of sandflies in the city of Najaf, the active local focus of leishmaniasis, also the molecular approach presented in this work is the first one developed for *Phlebotomine* and *Sergentomyia*.

The geographical distribution of cases, risk factors and disease occurrences are not documented yet. In spite of the increasing number of diagnosed cases, there is no regular record of these cases. Public health measures, such as case detection, treatment, the control of sandflies and health education can be effective in controlling the disease.[4]

Parvizi and Amirkhani[5] reported that the molecular epidemiology has become an essential tool to define the elements of a transmission cycle, and to identify the possible sources of infection.

Al-Ajmi et al.,[6] stated that the molecular identification is a valuable approach for determining the incidence in unchecked regions.

In this work, we inspected the *Phlebotomus spp.* as the most important vectors of leishmaniasis, in addition to *Sergentomyia spp.*, in the study areas. Phylogenetic tree of different *Phlebotomine sp.* showed that each species is much related to the same species reported as reference species in the GenBank. In addition, these parasites had been diagnosed from clinical specimens, in other studies in the same areas.

Al-Huchaimi et al.,[7,8] revealed that both *L. major* and *L. tropica* were the causative agents of cutaneous leishmaniasis in Najaf, and with existing cases showing cutaneous leishmaniasis in the area and the *Leishmania* isolated, *P. papatasi* and *P. sergenti* suspected to be the main vector of cutaneous leishmaniasis in Najaf. Thus, before planning any control measure against *Leishmania* vectors, a study should be performed to establish the baseline susceptibility to represent insecticides.

Al-Samarai and Al-Obaidi[9], indicated that the cutaneous leishmaniasis is epidemiologically unstable. Jarallah[10] reported that although cutaneous leishmaniasis cases have been reported in Iraq, the epidemiological and specification have not been well-documented. Al-Hamdi et al.,[11] stated that cutaneous leishmaniasis is endemic in Iraq.

Al-Ajmi et al.,[6] mentioned that both *L. major* and *L. tropica* are identified from *P. papatasi* and *P. sergenti*, respectively, using the semi-nested PCR method against kDNA and ITS1PCR-RFLP in Al-madinah Al-munawarah province of Saudi Arabia, in consequence, identifications of both sandfly and *Leishmania spp.* are of great significance for predicting the prevalence of the disease in endemic areas, and also help in designing new strategic programs, to limit the spread of such serious vectors.

Different populations of the same species of sandflies could differ in their transmissibility potential, and also different sandfly species of the same species of *Leishmania* could have different impact on strain virulence.[5]

Consistent with what has been observed by Maia et al.,[12] the role of *Sergentomyia* in the transmission of *Leishmania* parasites becomes noticeable, because *L. major* and *L. tropica* have been detected in this sandfly by molecular methods.

Parvizi, and Ready,[13] reported that one of the major obstacles for the control is the detection and identification of *Leishmania* parasite in vectors and reservoirs. The incidence may increase with little warning if the vector of sandflies is present.

In Iran, Parvizi and Paul[14] indicated the role of sandflies in the virus transmission, therefore it is essential to appreciate the discrimination of sandfly vectors because it shows where the vectors are coming from.

Future Studies

1. To understand the ordinary activities of *Leishmania spp.* in study areas, further studies needed to understand the vector and reservoir hosts for this parasite.
2. These findings so far required are the starting point and further investigations of the role of sandflies of the genus *Sergentomyia*, to clarify the transmission of leishmaniasis.

Conflict of Interest

None

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