

GENETIC DIVERSITY OF PHLEBOTOMUS PAPATASI POPULATIONS IN TWO DIFFERENT GEOGRAPHICAL **REGIONS IN EGYPT**

A thesis submitted to the Department of Entomology, Faculty of Science, Ain Shams University

For the award of Ph. D. degree in Entomology

By Noha Mohamed Abd El-Badiea

(M.Sc. in Entomology)

SUPERVISORS

Prof. Dr. Magdi Gebril Shehata

Faculty of Science Ain Shams University Prof. Dr. Nadia Mohamed Lotfy.

Professor of Medical Entomology Professor of Medical Entomology, Faculty of Science, Ain Shams University.

Dr. Doaa Elsayed Abd El- Kareem Soliman.

Lecturer of entomology, Faculty of Science, Ain Shams University.

Cairo, 2015

Biography

Name: Noha Mohamed Abd El Badiea

Date and Place of birth: February 1, 1983, Zaqazeeq city,

Al-Sharqyia governorate, Egypt

Degree Awarded: M.Sc. (Entomology)

Department: Entomology

Faculty: Science

University: Ain Shams

Date of Graduation: May, 2004

Date of M.Sc. degree: January, 2010

Occupation: Researcher in the Research and Training

Centre, Ain Shams University.

Date of Registration for PhD Award: July, 2010

DEDICATION

This thesis is dedicated to my mother for her encouragement, inspiration and love devoted to me

To my dear Husband for sacrifices he made, continuous help and encouragement he offered, throughout my study period

Finally, to my lovely sons (Ali & Omar) for many hours they spend without their mother around

ABSTRACT

Phlebotomus papatasi (Scopoli) (Diptera: Psychodidae) is the main vector of *Leishmania major*; which is the cause of self-limiting cutaneous leishmaniasis in the Old World. This sand fly is found in houses, animal shelters, caves and rodent burrows. It has a large geographical range, which includes the Middle East and the Mediterranean regions. The internal transcribed spacer 2 (ITS 2) of rDNA and the cytocrome b (cyt b) gene of mtDNA were sequenced from two populations originating from Sinai and Alexandria. The query lengths of ITS2 was 455 bp in both populations with the proportion of AT greater than 69%. The ITS2 sequences reflect interspecific variation which affected the geographical distribution and this can affect the infectivity status and the disease transmission by the sand flies and may be used as a base for better control of the disease, while the query lengths of Cyt b gene was 442 bp which was rich with AT content with the proportion of AT greater than 75%. The results observation reflect that cyt b not affected by the geographical distribution, this give clue that it has no role in the infectivity and transmission of the disease by the sand fly and it can be used as genetic marker in differentiation between different types of Phlebotomus species. A phylogenetic study was carried out on the same populations, ITS 2 showed little variations within populations according to different localities. This is actually the reason for poor geographical structuring in the NJ tree. No clear phylogeographical pattern was observed in Cyt b gene for the populations because shared haplotypes were present at the external nodes or at different nodes. These Methods might represent useful tools for a molecular large scale screening of Phlebotomine sand fly species caught in areas where leishmaniasis is endemic, in order to plan appropriate epidemiological surveillance programs for both Leishmania spp. and their vectors.

ULTIMATE THANKS TO ALLAH

ACKNOWLEDGEMENT

Warmest thankfulness goes to **Prof. Dr. Magdi Gebril Shehata**, professor of Medical Entomology, Faculty of Science, Ain Shams University, for suggesting the research point, revising the manuscript and for his consistent scientific advice and guidance, encouragement and direct supervision from the initial phase of this work and throughout my task. His invaluable effort made possible the achievement of this work.

Heartfelt thanks, appreciation and warmest gratitude goes to **Prof. Dr. Nadia M. Lotfy,** professor of Medical Entomology, Faculty of Science, Ain Shams University, for her supervision, sincere assistant, supporting facilities offered during the investigation, continuous guidance and encouragement and revising the manuscript.

Special deep thanks to **Dr. Doaa Elsayed Abe El Kareem,**Lecturer of entomology, Faculty of Science, Ain Shams University,
for her scientific guidance and direct supervision design experiments. I
do appreciate her fruitful supervision during the thesis writing,
correcting the draft and her general encouragement throughout this
study.

I want to express my sincere thanks to the staff members and colleagues of Research and Training Center on Vectors of Diseases for providing the specimen sand flies and helping me in its rearing

Finally, deep thanks and appreciation to all the stuff members and colleagues of Entomology department, Faculty of Science, Ain Sham University, for their kind help and continuous cooperation.

LIST OF ABREVIATIONS

A	Adenosine
Alex	Alexandria
Вр	Base pair
C	Cymine
Cl	Cutaneous lieshmaniasis
cm	centimeter
COI	cytochrome c oxidase I
Cyt b	Cytochrome b
G	Guanine
L	Lieshmania
Lu	Lutzomyia
ITS2	Internal transcribed spacer 2
ML	maximum likelihood
Min	Minute (s)
Ml	millimeter
μl	Microliter
Mm	Millimeter
mt DNA	Mitochondrial DNA
NJ	neighbor- joining

<i>P</i> .	Phlebotomus
PCR	polymerase chain reaction
rDNA	Ribosomal DNA
RH	relative humidity
RTC	Research and Training Centre
T	Thymine
VL	visceral leishmaniasis
WHO	World Health Organization

LIST OF TABLES

	Page
Table (1): Nucleotides base composition of the ITS2	
sequence analysis for the two populations of P.	
papatasi	69
Table (2): Nucleotides base composition of the cyt b	
sequence analysis for the two populations of P.	
papatasi	72
Table (3): Identity of ITS2 in the two <i>P. papatasi</i>	
populations with the others from seventeen	
countries	77
Table (4): Identity of cyt b in the two P. papatasi	
populations with the others from eighteen haplotypes	
of eight countries	89
Table (5): Amino acid repeats of ITS2 sequence in	
the two <i>P. papatasi</i> populations	102

Table (6): Amino acid repeats of cyt b sequence in the	
two P. papatasi populations	106

LIST OF FIGURES

	Page
Fig. 1: Agarose gel electrophoresis of PCR products	
of ITS2 (rDNA) in female <i>P. papatasi</i> collected from	
Sinai and Alexandria	62
Fig. 2: A garose gel electrophoresis of PCR products	
of cyt b (mtDNA) in female P. papatasi collected	
from Sinai and Alexandria	63
Fig. 3: Chromatogram of direct sequence analysis of	
ITS2 in female <i>P. papatasi</i> from Sinai	67
Fig. 4: Chromatogram of direct sequence analysis of	
ITS2 in female <i>P. papatasi</i> from Alexandria	68
Fig. 5: Chromatogram of direct sequence analysis of	
cyt b gene in female P. papatasi from	

Sinai	70
Fig. 6: Chromatogram of direct sequence analysis of	
cyt b gene in female P. papatasi from	
Alexandria	71
Fig. 7: Pair-wise alignment of ITS 2 sequences of	
the two populations of female P. papatasi collected	
from Sinai and Alexandria	76
Fig. 8: Multi-alignment of ITS2 in the female <i>P</i> .	
papatasi from Sinai with the seventeen	
countries	78
Fig. 9: Multi-alignment of ITS2 in female <i>P</i> .	
papatasi from Alexandria with the seventeen	
countries	82
Fig. 10: Pair-wise alignment of <i>cyt b</i> sequences of the	
two populations of P. papatasi collected from Sinai	
and Alexandria	88

Fig. 11: Multi-alignment of cyt b sequences of

female P. papatasi collected from Sinai with the	
eighteen haplotypes of other P. papatasi	90
Fig. 12: Multi-alignment of <i>cyt b</i> sequence of female	
P. papatasi from Alexandria with the eighteen	
haplotypes of other <i>P. papatasi</i>	94
Fig.13: Alignment of amino acids sequence of ITS2	
gene in female P. papatasi. from Sinai	100
Fig.14: Alignment of amino acids sequence of ITS2	
gene in female <i>P. papatasi</i> . from Alexandria	101
Fig. 15: Alignment of amino acid sequences of cyt b	
gene in female P. papatasi from Sinai	104
Fig. 16: Alignment of amino acid sequences of <i>cyt b</i>	
gene in female <i>P. papatasi</i> from Alexandria	105
Fig. 17: Phylogenetic tree based on rDNA (ITS2)	
sequence data of female P. papatasi from Sinai and	
the selected other countries	109

Fig. 18: Phylogenetic tree based on rDNA (ITS2)	
sequence data of female P. papatasi from Alexandria	
and the selected other countries	
Fig. 19: phylogenetic tree based on mtDNA (cyt b)	
sequence data of female P. papatasi from Sinai and	
the selected countries	
Fig. 20: phylogenetic tree based on mtDNA (cyt b)	
sequence data of female P. papatasi from Alexandria	
and the selected countries	112

CONTENTS

	Page
I. INTRODUCTION	1
II. LITERATURE REVIEW	6
1. Identification of sand flies by using genetic markers	
around the world	6
1.1. Ribosomal DNA	6
1.2. Mitochondrial DNA	18
III. MATERIALS AND METHODS	44
1. Sand flies collection colonization	44
2. DNA Amplification	46
2.1. Extraction of DNA	46
2.1.1 Extraction by Gene JET TM Genomic DNA	
Purification Kit	47
2.1.2. Extraction by phenol: chloroform: Isoamyl alcohol	1 50
2.2. PCR Analysis	53
2.3. Agarose gel electrophoresis	54
B. Direct sequencing	58

4. Alignment and Phylogeny	59
IV. RESULTS	60