

**AL-QUDS UNIVERSITY**  
**Faculty of Graduate Studies**

**DIAGNOSIS OF CUTANEOUS LEISHMANIASIS USING  
RESTRICTION ANALYSIS OF KINETOPLAST DNA  
POLYMERASE CHAIN REACTION PRODUCTS**

**By**

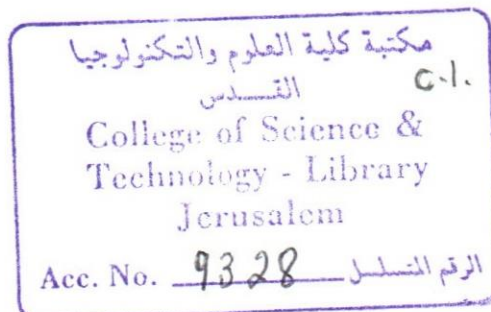
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# ENDORSEMENT

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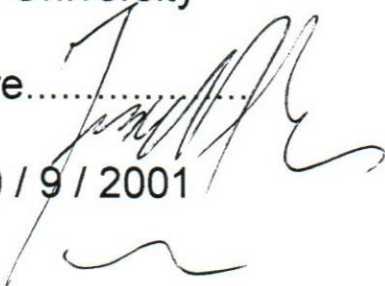
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## List of Abbreviations

|                            |   |
|----------------------------|---|
| A                          | Arava   |
| Bp                         | Base pair   |
| BPB                        | Bromo Phenol Blue                                     |
| BSA                        | Bovine Serum Albumin                                  |
| CL                         | Cutaneous Leishmaniasis                               |
| DCL                        | Diffuse Cutaneous Leishmaniasis                       |
| DMSO                       | Di Methyl Sulf Oxide                                  |
| EF                         | Excreted Factor                                       |
| EG                         | Egypt   |
| ELISA                      | Enzyme-Linked Immunosorbent Assay                     |
| ET                         | Ethiopia  |
| FCS                        | Fetal Calf Serum                                      |
| HIV                        | Human Immunodeficiency Virus                          |
| IL                         | Israel  |
| IN                         | India   |
| IQ                         | Iraq  |
| ITS1                       | Internal Transcribed Spacer                           |
| J                          | Jericho   |
| JO                         | Jordan  |
| J.V                        | Jordan Valley   |
| K.A                        | Kfar Adumim   |
| KDNA                       | kinetoplast DNA                                       |
| KE                         | Kenya   |
| Laet                       | Leishmania aethiopica                                 |
| Li                         | Leishmania infantum                                   |
| Lm                         | Leishmania major                                      |
| LR                         | Leishmaniasis Recidivans                              |
| LRC                        | Leishmania Reference Center                           |
| Lt                         | Leishmania tropica                                    |
| MCL                        | Muco Cutaneous Leishmaniasis                          |
| MW                         | Molecular Weight                                      |
| N                          | Negev   |
| OD                         | Optical Density                                       |
| PBS                        | Phosphate Buffered Saline                             |
| RFLP                       | Restriction Fragment Length Polymorphism              |
| PCR                        | Polymerase Chain Reaction                             |
| Ph. Papatasi               | Phlebotomus Papatasi                                  |
| PPIP-PCR<br>Chain Reaction | Permissively Primed Intergenic Polymorphic Polymerase |
| P. Obesus                  | Psammomys Obesus                                      |
| PS                         | Palestine   |
| Rpm                        | revolutions per minute                                |
| SL                         | Spliced Leader  |
| SD                         | Sudan   |
| SN                         | Sanegal   |
| SU                         | Soviet Union  |
| SSU rRNA                   | Small Subunit Ribosomal RNA                           |
| T                          | Tiberias  |
| TN                         | Tunisia   |

|     |                           |
|-----|---------------------------|
| TR  | Turkish                   |
| U   | Uvda                      |
| UV  | Ultra Violet              |
| UZ  | Uzbekistan                |
| VL  | Visceral Leishmaniasis    |
| W.B | West Bank                 |
| WHO | World Health Organization |

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## ABSTRACT

Cutaneous leishmaniasis is generally characterized by skin lesions. Localized lesions that heal spontaneously over a few months are usually caused by *L. major*, with *Psammomys obesus* and *Meriones crassus* as the most important reservoir hosts. Lesions that may last up to several years are usually caused by *L. tropica*. Identification and characterization of *Leishmania* parasites from patients, as well as from reservoir hosts, is important for understanding the epidemiology of leishmaniasis. In addition, fast and accurate diagnosis is important for providing the correct patient care.

We have employed a kinetoplast DNA (kDNA) based polymerase chain reaction (PCR) technique for direct diagnosis of *Leishmania* parasites from tissue samples. The PCR reaction utilizes the Uni21/Lmj4 primer pair, originally designed to amplify the full *L. major* minicircle DNA. In fact, this primer pair can amplify all Old World *Leishmania* minicircles including *L. tropica*, *L. aethiopica*, and the *L. donovani* complex. As minicircle of the different species have similar sizes, ranging between 650 - 850 bp, we applied restriction endonucleases (Mbol, RsaI, BanII, HpaII, HaeIII). We optimized the method so that restriction enzyme analysis of kinetoplast DNA can aid in diagnosis and species identification. In this project, results show that RsaI, Mbol and HaeIII give different restriction patterns for all species and can discriminate between distant regional isolates of both *L. major* and *L. tropica*. Both standard and high fidelity Taq polymerases were used for amplifying the kDNA minicircle of leishmanial strains. Using the standard Taq polymerase, we obtained different restriction patterns for each Old World