

Genetic diversity and haplotype analysis of *Leishmania tropica* identified in sand fly vectors of the genera *Phlebotomus* and *Sergentomyia* using next-generation sequencing technology

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Abstract

Next-generation sequencing (NGS) was used to investigate the genetic diversity of *Leishmania tropica* in the sand fly vector, targeting the internal transcribed spacer 1 (ITS1) of the genus *Leishmania*. Bioinformatics analyses were conducted using Galaxy, MEGA version X, DnaSP ver. 6.12.03, and PopART 1.7 software for NGS analysis, phylogenetic tree, genetic diversity, and haplotype networking, respectively. A total of 307 engorged sand flies were trapped, with an overall *Leishmania* infection rate of 9.4 (29/307) and 6.8% by NGS and ITS1-PCR, respectively. Two *Leishmania*-infected sand fly genera were identified: *Phlebotomus* (10.2%, 26/254) and *Sergentomyia* (5.7% (3/53)). The phylogenetic tree showed two clusters, cluster I included the four study sequences along with 25 GenBank-retrieved DNA sequences. Cluster II consisted of three sequences from Iran and Pakistan. The genetic diversity analysis for the 29 *L. tropica* sequences showed high haplotype (gene) diversity index (Hd) (0.62 ± 0.07) but low nucleotide diversity index (π) (0.04 ± 0.01). Tajima's D, a neutrality test, is more negative in cluster I ($D = -2.0$) than in total population ($D = -1.83$), but both are equally significant ($P < 0.001$), indicating that observed variation in cluster I and whole population is less frequent than expected. The median-joining haplotype network produced a total of 11 active haplotypes. In conclusion, *L. tropica* from sand flies in Palestine is monophyletic that assembled in one main phylogroup and one haplotype.

Keywords: Genetic diversity; Haplotype analysis; *Leishmania tropica*; Next-generation sequencing; *Phlebotomus*; *Sergentomyia*.