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Research paper

Increased prevalence of human cutaneous leishmaniasis in Israel and the Palestinian Authority caused by the recent emergence of a population of genetically similar strains of *Leishmania tropica*Kifaya Azmi ^{a,b,1}, Lena Krayter ^{c,*1}, Abedelmajeed Nasereddin ^{a,d}, Suheir Erekat ^{a,b}, Lionel F. Schnur ^d, Amer Al-Jawabreh ^a, Ziad Abdeen ^a, Gabriele Schönian ^c^a Al-Quds Nutrition and Health Research Institute, Faculty of Medicine, Al-Quds University, Abu-Deis, West Bank, Palestine^b Department of Biochemistry and Molecular Biology, Faculty of Medicine, Al-Quds University, East Jerusalem, Abu-Deis, Palestine^c Institute of Microbiology and Hygiene, Charité University Medicine Berlin, Berlin, Germany^d Kuvin Centre for the Study of Infectious and Tropical Diseases, IMRIC, Hebrew University-Hadassah Medical School, Jerusalem, Israel

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ABSTRACT

Twelve unlinked microsatellite markers were used to determine the microsatellite profiles of 50 newly and 46 previously typed strains of *L. tropica* from various Israeli and Palestinian foci. Their microsatellite profiles were compared to those of 99 previously typed strains of *L. tropica* from 15 countries. Israeli and Palestinian strains of *L. tropica* fell into three different groups, one of which contained 75 of the 96 Israeli and Palestinian strains. This population separated from all the others at the first hierarchical level by Bayesian statistics and formed a distinct monophyletic group on applying genetic distance and allele frequency analyses. The second cluster contained ten Israeli strains from a specific focus north of the Sea of Galilee, which were previously shown to differ from all other strains of *L. tropica* in their serological, biochemical and molecular biological parameters. This cluster was closely related to clusters comprising strains of *L. tropica* from Africa. Four Israeli and five Palestinian strains fell into different genetic entities mostly related to strains from Asian foci of CL. Importation during numerous migrations of humans and, perhaps, infected reservoir animals in the past and, now, through modern travel is the most likely explanation for the existence of so many locally encountered genetic variants of *L. tropica* in the Israeli-Palestinian region. Geographical and ecological variation may play a role in expanding the genetic heterogeneity once given importations had become established in different foci. Currently, one population is expanding in the area comprising almost all of the Palestinian and Israeli strains of *L. tropica* isolated since 1996 and investigated in this study, which differ clearly from all other strains of whatsoever origin. This population seems to result from the re-emergence of a previously existing genotype owing to environmental changes and human activities.

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1. Introduction

In Israel and the Palestinian Authority, two indigenous species of *Leishmania* (Kinetoplastida: Trypanosomatidae), *L. major* and *L. tropica*, cause most of the human cases of cutaneous leishmaniasis (CL). A third indigenous species, *L. infantum*, is the main cause of human cases of visceral leishmaniasis (VL) however, recently the first four cases of

human CL caused by *L. infantum* were reported in the Israeli-Palestinian region (Azmi et al., 2012b).

Originally, the species *L. major* and *L. tropica* in the Israeli-Palestinian region differed in their distribution, depending on differences in their ecological habitats and modes of transmission. Most of the human cases of CL diagnosed in the study area were caused by *L. major* and these cases occurred mainly in the Jordan and Arava Valleys and in foci along the Western Negev. CL caused by *L. major* is a zoonotic disease and the distribution of the parasites was governed primarily by the need of soft soil for burrowing by the desert rodents, mainly *Psammomys obesus*, that serve as the animal reservoir. However, *L. tropica* was distributed more widely and mainly in the hilly and mountainous regions. Human cases of CL caused by *L. tropica* were sparse and it was considered to be anthroponotic (reviewed, Jaffe et al., 2004). More recent field studies carried out in Israel have suggested that some of the

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human cases of CL caused by *L. tropica* are zoonoses with rock hyraxes (*Procavia capensis*) serving as the animal reservoir (Jacobson et al., 2003; Svobodova et al., 2006).

Epidemiological surveys and field studies carried out from the late 1990s onward have documented a very significant increase in the number of human cases of CL caused by *L. tropica* and their wider geographical distribution throughout the central area of the Israeli-Palestinian region (Al-Jawabreh et al., 2004; Azmi et al., 2012a, 2012b; Schnur et al., 2004; Singer et al., 2008) and the emergence of new foci on the western side of the Sea of Galilee by the City of Tiberias and in the Lower Galilee north of this 'sea' (Fig. 1) (Svobodova et al., 2006; Vinitsky et al., 2010). Based on records for the years 2002 to 2009, many human cases of CL caused by *L. tropica* were recorded in the Jenin District, with an average annual incidence of 23.0 per 100,000 inhabitants (Azmi et al., 2012b). In some cases, the increase in the number of cases coincided with an increase in the numbers of rock hyraxes (*P. capensis*) close to human habitations, especially where the cases have occurred. In fact, a number of the hyraxes caught in the northern foci close to the Sea of Galilee were shown to be positive for DNA from *L. tropica* (Jacobson et al., 2003; Svobodova et al., 2006).

L. tropica is a very heterogeneous parasitic species associated with a broad and quite complex clinical spectrum of disease ranging from simple CL presenting as either single or multiple lesions to relatively rare cases of leishmaniasis recidivans (LR); rarer cases of visceral leishmaniasis (VL), including some that have also displayed post kala azar dermal leishmaniasis (PKDL) after treatment; and even an occasional case of oro-nasal and naso-pharyngeal leishmaniasis (Azmi et al., 2012a; Magill et al., 1993; Sacks et al., 1995; Schnur et al., 1981; Schnur and Greenblatt, 1995). Many previous studies have exposed the considerable serological (Azmi et al., 2012a; Jacobson et al., 2003; Jaffe et al.,

1990; Schnur et al., 2004), biochemical (Azmi et al., 2012a; Le Blancq and Peters, 1986; Mebrahtu et al., 1992; Nimri et al., 2002; Pratlong et al., 1991; Rioux et al., 1990) and genetic heterogeneity (Azmi et al., 2012a, 2013; Krayter et al., 2014a; Schonian et al., 2001) of this species.

In an earlier application of multilocus microsatellite typing (MLMT) (Schwenkenbecher et al., 2006), 21 independent microsatellite markers were used on 48 strains of *L. tropica* from different Israeli and Palestinian foci that fell into three distinct clusters of strains, named I, II and IV. Their 'cluster I' comprised most of the strains of *L. tropica* from the different Israeli foci and those in the Palestinian foci in Jenin and Jericho Districts. Their 'cluster IV' comprised strains of *L. tropica* from the focus of human CL north of the Sea of Galilee, where, in contrast to other foci, human CL caused by *L. tropica* is transmitted mainly by female sand flies of the species *Phlebotomus arabicus* and less so by *P. sergenti* known to be the usual vector of *L. tropica*. Their 'cluster II' comprised many other strains of *L. tropica* of various sources and geographical origins, and, interestingly, two older Israeli strains isolated in 1949 and 1990, and four more recently isolated Palestinian strains of *L. tropica* from the Jenin District. The existence of, at least, three different genetic categories of Israeli and Palestinian strains of *L. tropica* based on microsatellite profiles was paralleled by the different strains' distinctive antigenic, isoenzyme profile and kDNA attributes (Azmi et al., 2012a, 2013; Jacobson et al., 2003; Schnur et al., 2004).

So far, MLMT has proved to be the most discriminating means for identifying and distinguishing strains of *L. tropica*. However, the use of 21 markers is expensive, labour-intensive and time-consuming. To minimize these limitations, the set of microsatellite markers has been reduced from 21 to 12 and proven to have the same discriminatory power in population genetic studies (Krayter et al., 2014b). Here, the reduced set of microsatellite markers was applied to the genetic characterization of more recently isolated Israeli and Palestinian strains of *L. tropica*, mainly from human cases of CL but also from sand fly vectors and animal hosts, whose microsatellite profiles were compared to those of the previously isolated strains of *L. tropica* from the Israeli-Palestinian region and, also, to strains of *L. tropica* from various sources and of many different geographical origins.

2. Materials and methods

2.1. Ethical clearance

The strains of *L. tropica* from human cases were isolated during routine diagnosis with no unnecessary invasive procedures and with written and/or verbal consent recorded at the time of clinical examination. Case data were encoded upon sample collection to confer anonymity. The Ethical Committees of Al-Quds University and the Hebrew University-Hadassah Medical School, Jerusalem, approved all activities involving human subjects.

2.2. Parasite strains

Fifty strains of *L. tropica*, 31 Palestinian and 19 Israeli, were studied, all of which were isolated from human cases of CL between 2000 and 2011, except for two of the Israeli ones, one of which came from a hyrax of the species *Procavia capensis*; the other from a female sand fly of the species *P. sergenti*. The provenances of these test strains are listed in Table S1 in bold print.

Their microsatellite profiles were compared to those of 145 strains of *L. tropica*, most of which had been typed previously (Krayter et al., 2014a, 2014b; Schwenkenbecher et al., 2006). The strains used for comparison were isolated mainly from human cases of CL and a few from human cases of VL, female sand flies and hyraxes, 21 of which came from Israeli foci and 25 from Palestinian foci, and the rest from other various geographical locations (Table S1, Fig. 1). Microsatellite profiles of eight strains of *L. aethiopica* (Table S1) were also included for comparison (Krayter et al., 2015).

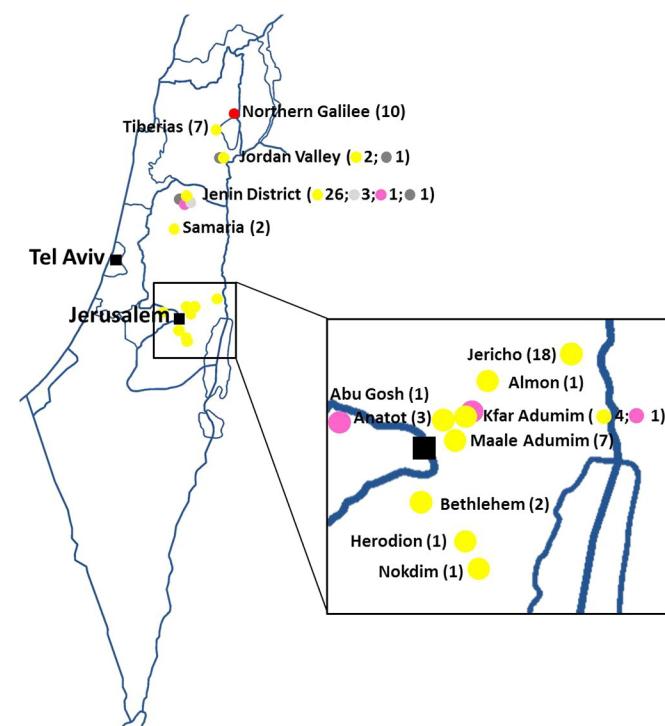


Fig. 1. Distribution of strains of *L. tropica* in the Israeli-Palestinian region. Map showing the geographical distribution of the Israeli and Palestinian strains, indicating their assignment to the different populations inferred by Bayesian statistics. The colours in the circles represent the different populations. The numbers in parentheses are the number of strains of the given populations. Two strains from Jenin had shared membership and have been excluded from the figure as have two other strains owing to their unspecified geographical origins. This map is a modification based on the one shown at <https://commons.wikimedia.org/w/index.php?curid=491722> under a CC BY 2.5 license by Golbez.

2.3. Microsatellite amplification

The twelve unlinked co-dominant microsatellite markers applied in earlier studies (Krayter et al., 2014a, 2014b) were used in this study.

The sequences containing microsatellites were amplified in reaction volumes totalling 25 µl, using the PCR-Ready Supreme mix (Syntezza Bioscience, Jerusalem, Israel) in a Gene Amp PCR-system 9700 thermal cycler (Applied Biosystems, CA, USA). PCRs were performed with fluorescence conjugated forward primers (Fam, Hex and Ned), and started by denaturing for 5 min at 95 °C, followed by 35 amplification cycles of 30 s at 95 °C, 30 s at the marker-specific annealing temperature (AT, see Krayter et al., 2014b) and 45 s at 72 °C, ending with a final period of extension of 7 min at 72 °C.

2.4. Fragment analysis

A quality check was performed by running the PCR products on 3% agarose gels. Three of those products at a time, each one labelled with a different fluorescence dye, were put together in one tube and sent for fragment analysis to the Center for Genomic Technologies at the Hebrew University of Jerusalem. This was done with the automated fragment analysis tool, using the ABI PRISM 3730xl DNA Analyzer genetic analysis system. GeneMapper software version 3.7 (Applied Biosystems, Foster City, USA) and peak scanner (software V.1, Applied Biosystems) were used for the subsequent analysis of the peaks.

To compare fragment lengths produced in different laboratories and using different sequencers, the data had to be normalized. Therefore, strain MHOM/PS/2001/ISL590, whose microsatellite loci had already been sequenced (Schwenkenbecher et al., 2004), was included in each PCR and in each fragment analysis run as a standard for size. The fragment lengths were compared to those of the reference strain and their virtual fragment sizes were calculated by determining the repeat numbers of each strain compared to the reference strain and multiplying them by the size of the microsatellite (di- or tri-nucleotide repeat) and, finally, adding the size of the flanking regions. The resulting virtual fragment sizes were recorded in an excel-input file.

2.5. Population genetic analyses

The excel-input file was converted into the appropriate input files for the following population genetic programs, using MSA software version 4.05 (Dieringer and Schlötterer, 2003) and CONVERT 1.31 (Glaubitz, 2004).

The population structure was inferred by Bayesian clustering implemented in STRUCTURE software version 2.3.4 (Pritchard et al., 2000). Based on allele frequencies, it identifies genetically distinct groups and estimates each strain's membership to the groups. The length of the burn-in period and the number of Markov Chain Monte Carlo Repeats were set to 20,000 and 200,000, respectively. DeltaK gives an estimate of the most probable number of distinct populations and was also calculated with this software, using 10 replicate runs for each K (Evanno et al., 2005).

Factorial correspondence analysis (FCA) is a multidimensional statistical method to evaluate the number of genetic groups. This algorithm is implemented in Genetix 4.05 (Belkhir et al., 1996–2004) and was applied to the accumulated data.

POPULATIONS software version 1.2.32 was used to calculate the genetic distances, using the Chord distance settings (Cavalli-Sforza and Edwards, 1967) (<http://bioinformatics.org/~tryphon/populations/>). The Neighbour Joining (NJ) tree was visualized with MEGA 6 (Tamura et al., 2013).

Hybridisation, horizontal gene transfer and recombination were investigated in a phylogenetic network that resulted from analysis using SplitsTree 4.12.8 (Huson and Bryant, 2006).

Genetic distances between populations (F_{ST}), mean number of alleles (A), observed (H_o) and expected (H_e) heterozygosity, and the

inbreeding coefficient (F_{IS}) were calculated, using MSA 4.05 and GDA 1.1 (Lewis and Zaykin, 2001).

3. Results

All of the 64 newly typed strains, of which 50 had been isolated in Israeli and Palestinian foci of CL and 14 were of different geographical origin, possessed the 12 microsatellite markers, which were amplified and analysed successfully without exception. Twenty-four of the newly typed strains, 17 Palestinian and seven Israeli, shared the microsatellite profile *LtromS* 041 previously found in three strains of *L. tropica* isolated from cases of CL in Jericho. The profile *LtromS* 042 previously seen in a Palestinian strain was also displayed by two of the newly typed strains, one Palestinian and one Israeli. In three other cases, profiles *LtromS* 063, *LtromS* 065 and *LtromS* 089, each of two newly typed strains shared the same microsatellite profile. The remaining 18 new Israeli and Palestinian strains had unique microsatellite profiles (Table S1).

The number of alleles per locus ranged from 3 (GA6 and GA9n) to 20 (LIST7039) with a mean of 9.75 (Table 1). The observed heterozygosity (H_o) was lower than the expected heterozygosity (H_e) in all loci.

On applying the Bayesian clustering approach to all 203 strains of *L. tropica* and *L. aethiopica* and subsequent sub-structuring of the resulting populations, 76 of the Israeli and Palestinian strains, of which 44 were newly isolated, and the one from the Sinai Peninsula, Egypt, were separated from all other strains at the first hierarchical level, forming what was termed the 'Israel/Palestine population' (Fig. 2).

Re-running the data of all other strains, excluding those from the population 'Israel/Palestine', in a new Bayesian analysis produced two sub-populations: one encompassing strains from Asia termed the 'Asia sub-population'; the other comprising strains of *L. tropica* mainly from Africa, all the strains from the northern Galilean focus and the eight strains of *L. aethiopica*, and termed the 'Africa/Galilee sub-population'. Re-analysing this latter sub-population, using STRUCTURE, indicated six different clusters (Fig. S1) that were confirmed in all ten iterations. One of these clusters, termed the 'Northern Galilee cluster', encompassed ten Israeli strains, including two newly isolated ones, from the northern Galilean focus. Four Palestinian strains isolated in 2002, one isolated 1980 in Israel and nine from other Middle Eastern countries grouped together with Indian strains, forming the cluster 'Middle East/India'. Further analysis exposed three genetic groups in this cluster and indicated membership in all of them for two Palestinian strains, MHOM/PS/2002/20JnYM3 and MHOM/PS/2002/52JnYM18. The cluster 'old strains/Turkey' consisted of old strains of different geographical origins, three of them were Israeli, collected between 1949 and 1991, and one was Palestinian, isolated in 2002.

The population 'Israel/Palestine' was confirmed by all three subsequent analyses: NJ tree, Neighbour network and FCA. In the NJ tree, these strains formed a monophyletic entity (Fig. 3), one branch of

Table 1

Descriptive statistics by locus. A, number of alleles; H_o , observed heterozygosity; H_e , expected heterozygosity; F_{IS} , inbreeding coefficient (-1 = outcrossing, 0 = random mating, +1 = inbreeding).

Locus	A	H_o	H_e	F_{IS}
GA1	7	0.020	0.124	0.840
GA2	10	0.163	0.718	0.773
GA6	3	0.000	0.141	1.000
GA9n	3	0.347	0.482	0.281
LIST7010	11	0.208	0.837	0.752
LIST7011	11	0.015	0.782	0.981
LIST7027	14	0.406	0.817	0.504
LIST7033	11	0.035	0.667	0.948
LIST7039	20	0.131	0.824	0.841
LIST7040	15	0.355	0.703	0.496
4GTG	4	0.015	0.226	0.934
27GTGn	8	0.240	0.727	0.671
All	9.75	0.161	0.587	0.726

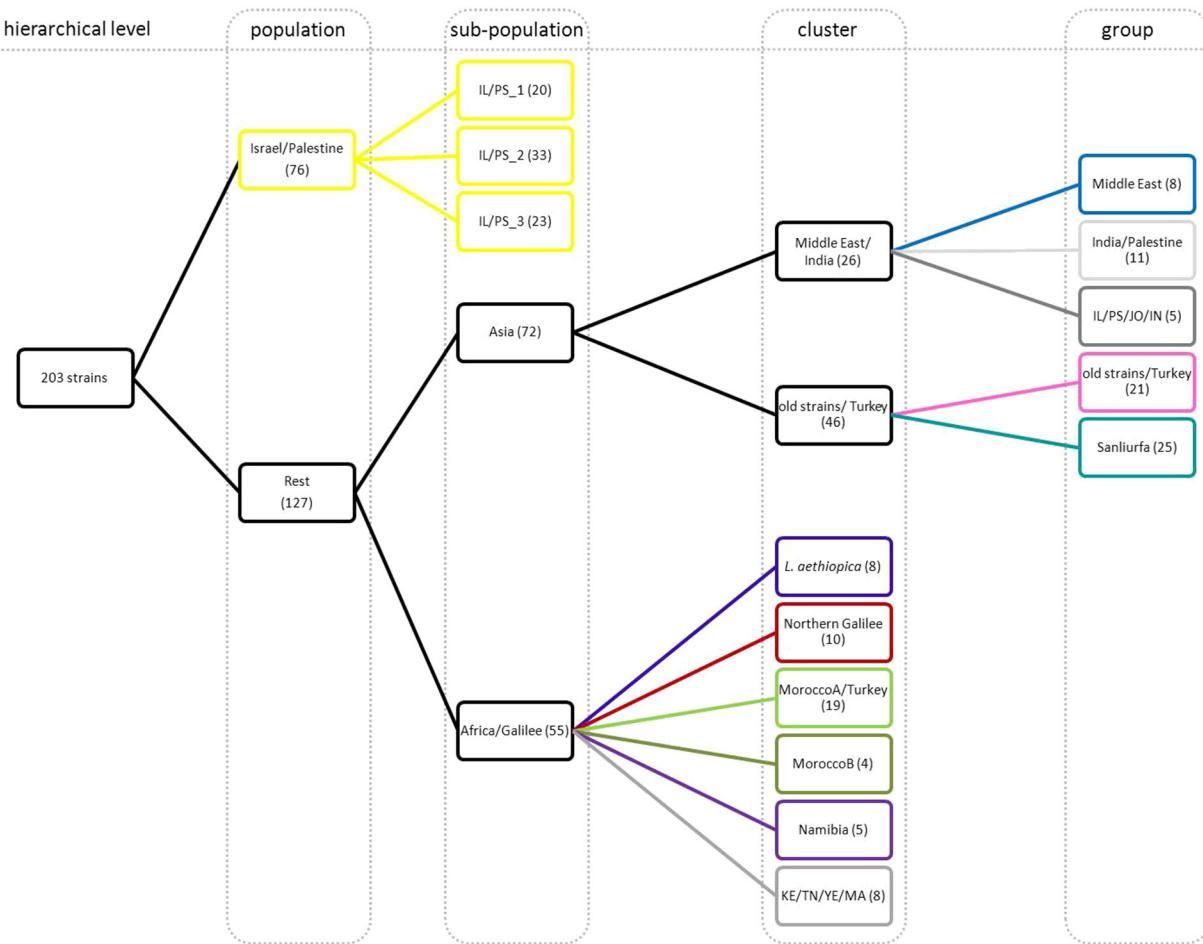


Fig. 2. Population structure inferred by Bayesian statistics. The hierarchical structure of the populations inferred by Bayesian statistics applied to the microsatellite profiles of all the strains of *L. tropica* and subsequent re-analysis of the inferred populations: each number in parentheses is the number of strains of that genetic entity; strains with shared membership in two or more clusters or groups have been omitted.

which contained 13 Palestinian strains isolated in 2002 and the one Egyptian strain from the Sinai Peninsula. The remaining 62 Israeli and Palestinian strains in this population did not aggregate in accordance with their geographical localization. In the network, the population 'Israel/Palestine' also appeared as a monophyletic entity separate from all other strains analysed in this study (Fig. 4), exhibiting only little conflicting branch positions as indicated by scarce cross connections. The FCA showed that the strains in the population 'Israel/Palestine' aggregated together and were distanced from all other strains of *L. tropica* (Fig. 5A). The three sub-populations of population 'Israel/Palestine' revealed by Bayesian statistics were not supported by any of the subsequent analyses.

The seclusion of the cluster 'Northern Galilee' was confirmed by the NJ tree, network and FCA (Figs. 3, 4 and 5A). The two newly typed strains isolated in that focus fitted perfectly into this entity. This cluster was more related to the African strains of *L. tropica* and closest to those from Namibia and Kenya.

In the NJ tree, the cluster 'Middle East/India', which also contained some of the Israeli and Palestinian strains of *L. tropica*, was monophyletic and formed two separate branches: one consisting of strains assigned to the group 'India/Palestine'; the other of strains assigned to the group 'Middle East' (Fig. 3). In the network, it was impossible to see a distinct separation of the subpopulations 'Asia' and 'Africa/Galilee' as well as of their clusters and groups (Fig. 4). The many cross-connections in the network indicated considerable conflicting branch positions for all strains except those forming the population 'Israel/Palestine'. This was in agreement with the FCA (Fig. 5B) where all strains of the cluster 'Middle East' were in close proximity to each other.

Table S2 shows that, on comparing all the phylogenetic entities with one another in all the possible combinations of pairs, almost every combination displayed a very great genetic difference according to their mean fixation indices (F_{ST}). Some of the F_{ST} values were not significant, indicated by an asterisk in Table S2.

The genetic entities containing the Israeli and Palestinian strains had mostly positive inbreeding coefficients ranging from 0.755 for the population 'Israel/Palestine' to 0.895 for the cluster 'Northern Galilee', albeit a negative one of -0.532 for the group 'India/Palestine' (Table 2).

4. Discussion

In this study of the variation in 12 unlinked microsatellite markers specific for the species *L. tropica*, strains of *L. tropica* from various Israeli and Palestinian foci, whether isolated recently and firstly studied here or isolated and typed previously, were consigned to three different genetic entities. Most of them ($N = 76$) were assigned to a strongly monophyletic population termed the population 'Israel/Palestine', which also included one Egyptian strain from the Sinai Peninsula. The strains of this population differed clearly from all the other strains from the Middle East, Asia and Africa. This was confirmed by all the methods applied. Strains isolated during a recent outbreak in the Israeli settlement of Ma'ale Adumim in the West Bank and from different villages in the Jenin District clustered perfectly in this most prominent population. The strong monophyletic nature of the population suggests that its members evolved from the same ancestral source and spread throughout the area. Regarding times of isolation, the Egyptian strain was the oldest strain in this population, having been isolated in 1990. All the

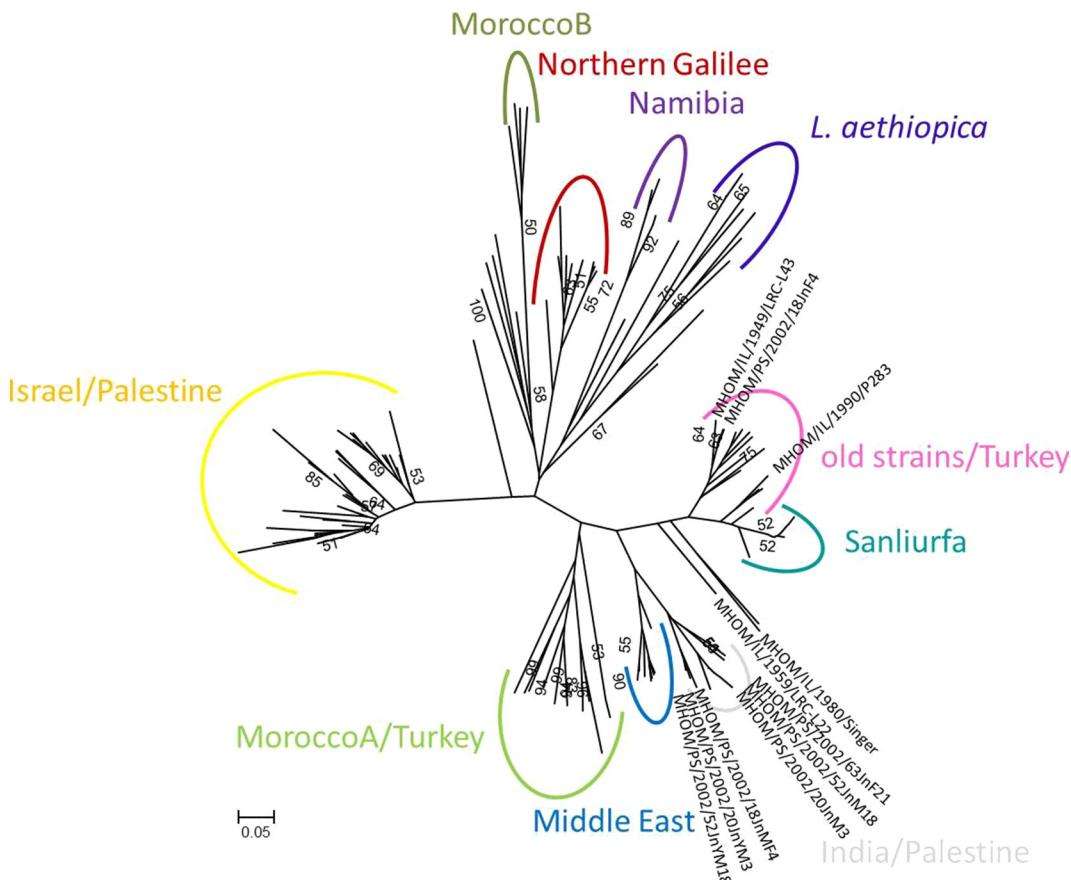


Fig. 3. The Neighbour Joining tree. The phylogenetic relationships among the strains are based on Chord distance estimates. Bootstrap values >50 are indicated at the nodes. The strains from Israeli and Palestinian foci are either found in "Israel/Palestine" or "Northern Galilee" or indicated by their WHO codes. Strain details are given in Table S1. The colours correspond with the results of the Bayesian statistics.

Israeli and Palestinian strains were isolated between 1996 and 2011. One can only speculate on whether the Egyptian strain or parasites genetically similar to it might have been the ancestral source of this population as microsatellite data do not enable studying phylogenetic evolution (Schonian et al., 2011). Leishmanial sequencing data encompassing the whole genome might resolve this.

The recent continual increase in the number of human cases of CL caused by *L. tropica* in different Israeli and Palestinian foci seems to be related to the appearance and spread of a population of genetically similar strains of *L. tropica* throughout the central area of the Israeli-Palestinian region. From the results presented here, the expansion of this population is, probably, not the result of the introduction of a new genetic variant of the parasite but of the re-emergence of a previously existing parasite owing to environmental changes, new construction and increased agriculture carried out by the human population.

This contrasted with the situation in the focus north of the Sea of Galilee. The ten Israeli strains of *L. tropica* collected from human cases of CL, female sand flies and a hyrax were clearly a separate and distinct genetic entity different from all the isolates of *L. tropica* from the other Israeli and Palestinian foci, not only by their microsatellite profiles but also regarding their biochemical, serological and molecular biological parameters (Azmi et al., 2012a, 2013; Jacobson et al., 2003; Schnur et al., 2004). In this focus, the parasites are transmitted mainly by the sand fly vector species *P. arabicus* and to a lesser extent by *P. sergenti*, the usual vector species of *L. tropica* in Israeli and Palestinian foci of CL (Jacobson et al., 2003; Schnur et al., 2004). So far, this new genotype seems to be restricted to this rather small area and has not yet been found among strains isolated in other Israeli and Palestinian foci, not even in those in close proximity. Interestingly, in all analyses undertaken, this group of strains clustered closely to African strains of *L. tropica*,

suggesting an African origin. Genetic distance analyses indicated that these strains were closest to *L. tropica* strains from Kenya and/or to two strains of '*L. killicki*' from Tunisia. Readers should note that more recent studies using different types of molecular analyses have shown that '*L. killicki*' belongs in the '*L. tropica*' complex and does not represent a separate species (Chaouch et al., 2013; El Baidouri et al., 2013; Pratlong et al., 2009; Schonian et al., 2001; Schwenkenbecher et al., 2006). This was confirmed by two studies applying MLST (Chaara et al., 2015a) and MLMT (Chaara et al., 2015b) on a larger number of strains of '*L. killicki*' from Tunisia, Algeria and Libya and of strains of *L. tropica* from Morocco despite clear differences between the two sets of strains. The authors suggested that strains of '*L. killicki*' emerged from strains of *L. tropica* by a single founder effect. This situation should be investigated further and also whether the parasites of the 'Northern Galilee' cluster described in this study might have evolved similarly.

Some of the Israeli and Palestinian strains were consistently consigned to the sub-population 'Asia' where they were distributed among three of its five groups. Three old Israeli strains isolated in 1949, 1959 and 1990 and one Palestinian strain isolated in 2002 belonged to the group 'old strains/Turkey' together with other old strains of various geographical origins. One Israeli and one Palestinian strain fell into the group 'IL/PS/JO/IN' together with two Indian and one Jordanian strain. Another three strains from the Jenin District and eight from Bikaner City, India, formed the group 'India/Palestine'. This genetic separation of some of the strains isolated from human cases of CL recorded as coming from Israeli and Palestinian foci could be the result of the importation of these strains from foreign foci by local citizens travelling abroad and returning with infections acquired while abroad.

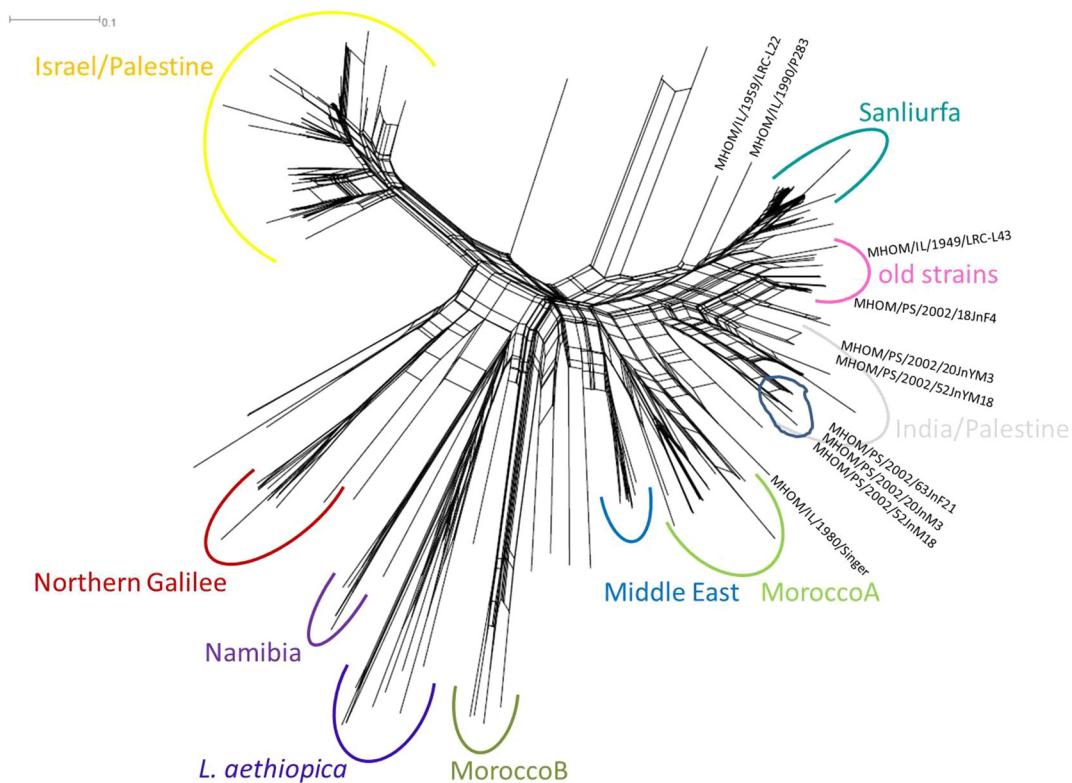


Fig. 4. The Neighbour Network. This Neighbour Network presents the phylogenetic relationships among the strains of *L. tropica* on applying SplitsTree 4. Cross-connections indicate probable reticulation events like hybridisation, recombination and horizontal gene transfer between the strains. The strains from Israeli and Palestinian foci are found in either the 'Israel/Palestine' or 'Northern Galilee' populations or indicated by their WHO codes. Strain details are given in Table S1. The colours correspond with the results of the Bayesian statistics.

Since the Israeli, and Palestinian regions combined cover a relatively small territorial area, the two regions are considered indistinguishable from each other in terms of the origins of the strains of *L. tropica* collected. Also, the WHO codes usually give the place of diagnosis of the human cases and do not necessarily reveal where the patients actually got infected. Owing to extensive travel and human migration from a great variety of countries into this area over past centuries and more so in recent times, the high degree of genetic variation among strains of *L. tropica* could be explained as the introduction of various strains of *L. tropica* independently into this area from different countries at different times.

In the past, the species *L. tropica* was thought to be solely anthroponotic. This, probably, is the case in many places in the geographical range of *L. tropica* and its vectors. However, more recently it has become clear that in some situations it seems that *L. tropica* is being transmitted zoonotically. In fact, DNA as well as whole parasites of *L. tropica* have been isolated from Namibian and Israeli rock hyraxes of the species *P. capensis* (Talmi-Frank et al., 2010a) and its DNA was also detected in blood meals of hyrax blood imbibed by female sand flies of the species *P. sergenti* (Valinsky et al., 2014). Hyraxes are the suspected reservoirs of *L. tropica* in the foci in central and northern Israel (Jacobson et al., 2003; Svobodova et al., 2006). The hyrax populations in these foci have increased in recent years, probably owing to human settlement activities in rural areas and the building of homes. This has brought the hyraxes and the parasites of *L. tropica* closer to human habitation. Whereas once human cases of CL caused by *L. tropica* were rare and were the result of humans visiting the sparse foci of these parasites, many cases are occurring now because, it seems, the parasites have been brought to the human population. *P. capensis* is distributed throughout sub-Saharan Africa except for the Congo Basin and Madagascar, extending from Namibia in southern Africa into Algeria, Libya and Egypt in North Africa and into the Middle East as far as northern Syria.

Therefore, hyrax migration into local foci could account for the introduction of strains of *L. tropica* in addition to human migration.

Very recently, a pet Pekingese dog from Ma'ale Adumim, Israel, was described as a case of muco-cutaneous leishmaniasis (MCL) caused by *L. tropica* (Baneth et al., 2014). Further, golden jackals (*Canis aureus*) and red foxes (*Vulpes vulpes*) caught in Israel tested positive for DNA from parasites of *L. tropica*, suggesting they were carrying such infections (Talmi-Frank et al., 2010b). However, the role that domestic dogs and wild canids play in the transmission of *L. tropica* is uncertain and needs further investigation. Even so, these could be a further source of the introduction of strains of *L. tropica* into the geographical region under discussion.

In conclusion, the Israeli and Palestinian strains of *L. tropica* showed substantial genetic variation. The region has been a land bridge between Europe, North and East Africa and Western Asia and is also geographically and ecologically highly diverse. The vast majority of Palestinian and Israeli strains of *L. tropica* were, however, assigned, by all the methods applied, to a strongly monophyletic population termed 'Israel/Palestine'. It was clearly separated from all other strains from this and other geographical regions and, most probably, the result of the re-emergence of a previously existing parasite owing to environmental changes and human activities. Ten Israeli strains from the northern side of the Sea of Galilee differed from the main population not only by their MLMT profiles but also with respect to their transmission cycle and by biochemical, serological and molecular biological parameters. They were found to be more closely related to African strains of *L. tropica* and an African origin has been suggested. The remaining 11 Israeli and Palestinian strains grouped together with Asian strains of *L. tropica*, namely Turkey and India, but the assignment was, however, not consistent with all the types of analyses used. These highly diverse strains of *L. tropica* could have been introduced independently into Palestinian/Israeli area from different countries owing to human or reservoir host migration.

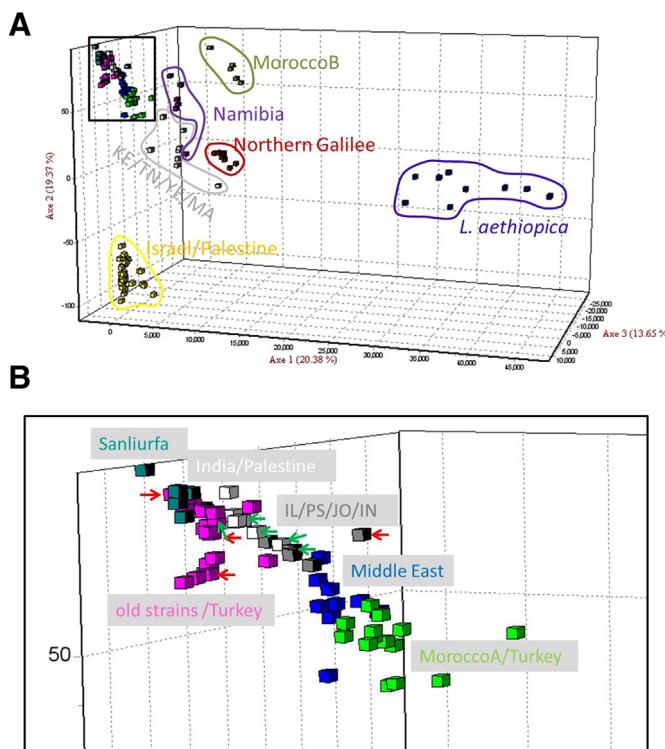


Fig. 5. Factorial correspondence analysis. FCA was applied to the strains following their assignment to the populations generated through Bayesian clustering: A, includes all the strains in the analysis and each of the genetic entities inferred by the Bayesian statistics is encompassed by a different colour; B, is an enlarged and clearer view of the entity encompassed by the black rectangle in Fig. 4A where the Israeli and Palestinian strains are indicated by the red and green arrows, respectively.

Competing interests

The authors declare that they have no competing interests.

Author contributions

KA and LK contributed equally to this study and should both be regarded as a senior author. KA, AN and LFS were involved in collecting the Palestinian and the Israeli strains. KA amplified the DNA samples extracted by SE and AN. KA and LK did the fragment analyses, LK carried out the population genetic analyses, LK and GS analysed and interpreted the phylogenetic results and wrote the manuscript together with LFS. AN, AA and ZA were involved in the design of the study.

Table 2

Descriptive statistics by population. A, number of alleles; H_o , observed heterozygosity; H_e , expected heterozygosity; F_{IS} , inbreeding coefficient (-1 = outcrossing, 0 = random mating, $+1$ = inbreeding).

Population	A	H_o	H_e	F_{IS}
Israel/Palestine	2.75	0.035	0.143	0.755
Middle East	1.67	0.333	0.268	-0.266
India/Palestine	1.75	0.402	0.269	-0.532
IL/PS/JO/IN	2.17	0.343	0.379	0.103
Old strains/Turkey	2.50	0.327	0.306	-0.070
Sanliurfa	1.33	0.318	0.170	-0.921
<i>L. aethiopica</i>	3.25	0.266	0.507	0.492
Northern Galilee	1.83	0.025	0.226	0.895
MoroccoA/Turkey	3.42	0.143	0.370	0.620
MoroccoB	1.83	0.118	0.278	0.624
Namibia	1.75	0.021	0.302	0.942
KE/TN/YE/MA	3.42	0.126	0.544	0.780
Mean	2.31	0.205	0.314	0.354

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.meegid.2016.07.035>.

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