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Original article

The paraphyletic composition of *Leishmania donovani* zymodeme MON-37 revealed by multilocus microsatellite typing

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Abstract

Multilocus microsatellite typing (MLMT) was employed to compare strains of *Leishmania donovani* belonging to the MON-37 zymodeme (MON-37 strains) from Cyprus and Israel to MON-37 strains from the Indian subcontinent, the Middle East, China and East Africa as well as strains of other zymodemes. The MLMT data were processed with a distance-based method for construction of phylogenetic trees, factorial correspondence analysis and a Bayesian model-based clustering algorithm. All three approaches assigned the MON-37 strains to different distantly related genetically defined subgroups, corresponding to their geographical origin. Specifically, the Kenyan, Sri Lankan and Indian MON-37 strains were genetically closer to strains of other zymodemes from the same regions than to MON-37 strains from other areas. MON-37 strains from Cyprus and Israel were clearly different not only among themselves, but also compared to all the other MON-37 strains studied and could, therefore, be autochthonous. This study showed that the zymodeme MON-37 is paraphyletic and does not reflect the genetic relationship between strains of different geographical origin.

Keywords: *Leishmania donovani*; Zymodeme MON-37; Microsatellites; Genotyping; Cyprus

1. Introduction

Strains of *Leishmania donovani* belonging to the MON-37 zymodeme (MON-37 strains) were isolated from cases of human visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) from widely separated locations of the Old World. They had caused VL in northwest India [1], Ethiopia [2], Morocco [3] and Israel [4], and VL and Post-kala-azar dermal leishmaniasis (PKDL) in Kenya [5]. Parasites had also been isolated from *Phlebotomus martini*, a proven vector for *L. donovani* in Kenya and Ethiopia [2,5]. During the past five years over 600 leishmaniasis cases were reported from various regions in Sri Lanka none of which were VL [6]. Here, MON-37 strain parasites were isolated from human CL [7]. In Himachal Pradesh in the Western Himalayas in India, a strain isolated from a VL patient proved to be a MON-37 strain of *L. donovani* [1]. This focus is endemic for human CL with an increasing number of cases, 810 since the first one recorded in 1988 [8,9]. In addition, 29 sporadic cases of VL, mostly indigenous, were reported from this area. The causative agents of CL and VL were either *L. donovani/Leishmania infantum* or/and *Leishmania tropica*. Microsatellite analysis had shown that the MON-37 strains of *L. donovani* from Sri Lanka and

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India form a distinct genetic cluster which is most closely related to a genetic cluster of *L. donovani* strains belonging to the MON-2 zymodeme from India, Bangladesh and Nepal. The cluster is genetically clearly different from the MON-37 strains of Kenya [10,11]. Human cases of VL and CL caused by MON-37 strains had recently been reported from Cyprus where only canine leishmaniasis had been known before [12]. This appears to be the first report of VL and CL caused by *L. donovani* sensu stricto in Europe.

MON-37 strains were not only isolated in classical foci of VL caused by *L. donovani* but also in regions where *L. infantum* has always been endemic such as Cyprus, Israel and Morocco. The most plausible explanation for VL in Morocco is a history of travels to areas where *L. donovani* is endemic. This was excluded for the VL cases in Israel and Cyprus. Four definitively locally acquired adult cases of VL had been recorded in Israel since 1978 with large time intervals between their occurrences. Two of them were caused by MON-37 strains [4]. This suggests that, even if these strains were imported into Israel, the parasite did not circulate in the population which is in contrast to the situation in Cyprus where six cases occurred within a 2–3 years period [12]. In addition, the cases were dispersed in three of the five governmental prefectures that fall into distinct and relatively distant bio-geographical regions. Indigenous competent putative vectors are present in Cyprus. The most probable scenario is that MON-37 strains were imported and established with infected immigrants from endemic countries who acted as primary reservoir for the parasites. However, the six Cypriot cases could also have been autochthonous.

We report here the comparison by multilocus microsatellite typing (MLMT) of MON-37 strains from Cyprus with strains from other parts of the Eastern Mediterranean and with MON-37 strains and strains of various other zymodemes from the Indian subcontinent, the Middle East, China and East Africa. MLMT was chosen for this analysis because of its proven highly discriminatory power in typing strains for population genetic studies on *Leishmania* parasites [11,13–15]. The 14 microsatellite markers used here were developed for studying strains of the *L. donovani* complex and are able to distinguish different geographical and genetic subgroups within the zymodemes. They are also able to identify putative hybrids and mosaic genotypes [15–17].

2. Materials and methods

2.1. Parasite strains and DNA extraction

Table 1 lists the 47 strains of *L. donovani* used in this study. Thirty-seven of these strains were of known association with 14 zymodemes. Thirty-nine of the strains had previously been typed by multilocus microsatellite profiling [11,14]. Fifteen of the 47 strains belonged to the MON-37 zymodeme and originated from 5 different countries on 3 continents. DNA was extracted from cultured promastigotes as described previously [18]. Multilocus microsatellite profiles of the 15 MON-37 strains were compared with each other and with those of the other 32 *L. donovani* strains.

2.2. Microsatellite typing

The 14 variable microsatellite markers Li 22-35, Li 23-41, Li 41-56, Li 45-24, Li 46-67, Li 71-5/2, Li 71-7, Li 71-33, Lm2TG, Lm4TA, TubCA, CS20, kLIST 7031 and kLIST 7039 were used in the present study as previously described [14]. Microsatellite-containing fragments were analyzed by capillary electrophoresis (SMB Services in Molecular Biology Berlin) with an automated ABI PRISM GeneMapper sequencer (Applied Biosystems).

2.3. Determination of genetic groups

Microsatellite-based genetic distances were calculated with the software packages MSA [19] and POPULATIONS (http://www.legs.cnrs-gif.fr/bioinfo/populations) by applying the Chord-distance measure [20]. Neighbour-joining (NJ) trees, including the test for confidence intervals by bootstrapping (100 replicates) based on the resulting distance matrix, were constructed with the programs POPULATIONS and MEGA [21].

The factorial correspondence analysis (FCA) implemented in the GENETIX software [22] was applied to visualise the genetic substructure in the data generated. This test places the individuals according to the similarity of their allelic state in three-dimensional space.

Strains of *L. donovani* were assigned to different genetic groups using the STRUCTURE 2.1 software which uses a Bayesian model-based clustering approach [23,24]. For each *K* value (number of genetic groups) between 1 and 10, 20,000 burn-in iterations followed by a run of 200,000 Markov Chain Monte Carlo iterations was performed.

3. Results

Nine of the 15 MON-37 strains had their own specific microsatellite profiles. The strains MHOM/CY/2006/CH33 and MHOM/CY/2006/CH35 from Cyprus shared the same profile, so did the strains MHOM/LK/2002/L60b and MHOM/LK/2002/L60c from Sri Lanka (Table 2). The Indian strains MHOM/IN/2003/LEM4537 and MHOM/IN/2003/LEM4527 that were isolated from the same patient also had the same profile. Sixteen percent of the allelic combinations of the 12 profiles were heterozygous presenting two repeats of different sizes. However, these heterozygous loci were distributed unevenly. Most of the profiles were generally homozygous but four strains, the Cypriot strains MHOM/CY/2006/CH32 and MHOM/CY/2006/CH34 from CL cases, the Israeli strain and the Kenyan strain from a sand fly, were heterozygous at 5–7 loci (Table 2).

The NJ phylogram (Fig. 1) constructed through a Chord-distance matrix calculated for the microsatellite profiles of the 15 MON-37 strains and the 32 strains of *L. donovani* of other zymodemes or of zymodemal affiliation, most from the Indian

Table 1
The strains of *Leishmania donovani* studied: CL = cutaneous leishmaniasis; VL = visceral leishmaniasis; CanL = canine leishmaniasis, PKDL = post-kala-azar dermal leishmaniasis; *Ph.* = *Phlebotomus*. The genetic groups 1–8 are according to STRUCTURE ($K = 8$); the other designations are populations according to [11,14].

WHO code	Country	Zymodeme	Clinical condition	Genetic Group
MHOM/IL/1998/LRC-L740	Israel, Tel Mond	MON-37	VL	1
MHOM/IQ/1977/BUMM3	Iraq, Khaldiya al Anbar	MON-3, LON-43	VL	1
MCAN/IQ/1981/SUKKAR2	Iraq, Wasit Shuhaimiya	MON-3, LON-43	CanL	1
MHOM/CN/????/Wangjie1	China	MON-35	VL	1
IMAR/KE/1962/LRC-L57	Kenya	MON-37	*Ph. martini*	2 and KE/IN2
MHOM/KE/1983/NLB 189	Kenya	MON-37	PKDL	2 and KE/IN2
MHOM/KE/1985/NLB 323	Kenya	MON-37	VL	2 and KE/IN2
MHOM/KE/1973/MRC74	Kenya	MON-2	VL	2 and KE/IN2
MHOM/KE/1955/LRC-L53	Kenya	MON-36	VL	2 and KE/IN2
MHOM/IN/1954/SC23	India	MON-38	VL	2 and KE/IN2
MHOM/KE/????/NLB-065	Kenya	LON-46	VL	2 and KE/IN2
MHOM/KE/1984/NLB-218	Kenya	Unknown	PKDL	2 and KE/IN2
MHOM/IN/1954/LRC-L51p[a]	India, Calcutta	Unknown	VL	2 and KE/IN2
MHOM/IN/1954/LRC-L51[a]	India, Calcutta	Unknown	VL	2 and KE/IN2
MHOM/SD/1992/51-band	Sudan, Gedaref	MON-30	VL	3 and SD/ET1
MHOM/SD/1993/762L	Sudan, Gedaref	MON-30	VL	3 and SD/ET1
MHOM/SD/1962/LRC-L61	Sudan	MON-82	VL	3 and SD/ET1
MHOM/SD/1993/AEB	Sudan, Gedaref	MON-82	VL	3 and SD/ET1
MHOM/SD/1968/1S	Sudan	Unknown	VL	3 and SD/ET1
MHOM/SD/1975/LV139	Sudan	Unknown	CL	3 and SD/ET1
MHOM/IN/1980/DD8	India	MON-2, LON-41	VL	4 and IN1
MHOM/IN/1996/THAK35	India, Bihar	MON-2	Unknown	4 and IN1
MHOM/IN/????/DEVI	India, Bihar	MON-2	VL	4 and IN1
MHOM/IN/2001/BHU20140	India, Bihar	Unknown	VL	4 and IN1
MHOM/IN/2002/BHU1	India, Bihar	Unknown	VL	4 and IN1
MHOM/IN/2002/BHU2	India, Bihar	Unknown	VL	4 and IN1
MHOM/CY/2006/CH32[b]	Cyprus, Paphos	MON-37	CL	5
MHOM/CY/2006/CH33[b]	Cyprus, Paphos	MON-37	VL	5
MHOM/CY/2006/CH34[b]	Cyprus, Paphos	MON-37	CL	5
MHOM/CY/2006/CH35[b]	Cyprus, Paphos	MON-37	CL	5
MHOM/CY/2006/CH36[b]	Cyprus, Limassol	MON-37	VL	5
MHOM/IN/1983/CHANDIGARH	India, Himachal Pradesh	MON-37	VL	6 and IN3
MHOM/IN/2003/LEM4527[b]	India	MON-37	CL	6
MHOM/IN/2003/LEM4537[b]	India	MON-37	CL	6
MHOM/UA/1984/MARZ-KRIM (MHOM/SU/1984/MARZ-KRIM)	Ukraine, Crimea	MON-73	VL	6/5
MHOM/LK/2002/L60c	Sri Lanka	MON-37	CL	7
MHOM/LK/2002/L60b	Sri Lanka	MON-37	CL	7
MHOM/LK/2002/L59[b]	Sri Lanka	MON-37	CL	7
MHOM/SA/1981/Jeddah KA	Saudi Arabia, Jaizan	MON-31, LON-42	VL	8 and SD/ET2
MHOM/ET/????/HUSSEN	Ethiopia	MON-31, LON-42	VL	8 and SD/ET2
MHOM/SD/1993/GE	Sudan, Gedaref	MON-82	VL	8 and SD/ET2
MHOM/SD/1997/LEM3429	Sudan, Gedaref	MON-257	VL	8 and SD/ET2
MHOM/SD/1997/LEM3463	Sudan, Gedaref	MON-258	VL	8 and SD/ET2
MHOM/SD/1997/LEM3472	Sudan, Gedaref	MON-267	PKDL	8 and SD/ET2
MCAN/SD/2000/LEM3946	Sudan, Gedaref	MON-274	CanL	8 and SD/ET2
MHOM/IN/1961/L13	India	Unknown	PKDL	8 and SD/ET2

[a] Different lines of the same strain.
[b] Data from new MON-37 strains used in this study.

subcontinent or East Africa (Table 1), fall into the following three main clusters [11,14]: (1) India-1/Bangladesh/Nepal, (2) Sudan/Ethiopia with two sub-clusters and (3) Kenya/India-2. In addition, there is a fourth cluster with the two strains from Iraq and a single strain from China. None of the MON-37 strains grouped with the *L. donovani* strains of the Sudan/Ethiopia cluster, nor did they form a single distinct group in the tree. The Kenyan MON-37 strains clustered as Kenya/India-2 which also contained strains belonging to zymodemes MON-2, MON-36 and MON-38. The two clusters with the Sri Lankan and Indian MON-37 strains seem to be distantly related to the main Kenya/India-2 cluster. MHOM/UA/1984/MARZ-KRIM belonging to zymodeme MON-73 which was isolated from a human VL case of the Crimean peninsula around the same time as strain MHOM/IN/1983/CHANDIGARH was isolated from a human case of CL in Himachal Pradesh in northwestern India appeared to be closest to the Indian MON-37 strains. The Cypriot strains formed a clearly distinct cluster. The Israeli MON-37 strain appeared to be unique with an intermediate position in the tree and was more

Table 2
The multilocus microsatellite profiles of the 15 strains of *L. donovani* belonging to the zymodeme MON-37: VL = visceral leishmaniasis; CL = cutaneous leishmaniasis; PKDL = post-kala-azar dermal leishmaniasis; chr. = chromosomal location; n.d. = not done.

WHO code	Source and clinical condition	Lm2TG Chr.1	TubCA Chr.34	Lm4TA Chr.1	Li41-56 Chr.36	Li46-67 Chr.31	Li22-35 Chr.1	Li23-41 Chr.25	Li45-24 Chr.17	Li71-33 Chr.31	Li71-5/2 Chr.35	Li71-7 Chr.30	CS20 Chr.19	kLIST7031 Chr.10	kLIST7039 Chr.30
MHOM/CY/2006/CH33[a]	VL	9	10	12	10	6	6	11	14	11	9	8	11	8	20
MHOM/CY/2006/CH35[a]	CL														
MHOM/CY/2006/CH36	VL	9	10	12	10	6	6	11	15	11	9	8	11	8	20
MHOM/CY/2006/CH32	CL	9 + 10	10	12	7	6 + 9	6 + 17	11 + 21	11	11	8	8 + 13	11 + 19	11	19
MHOM/CY/2006/CH34	CL	9 + 10	10	12	7	6 + 9	6	11 + 21	11 + 14	11	9	8 + 13	11 + 19	8 + 11	19
MHOM/LK/2002/L60[b]	CL	9	9	10	18	6	14	24 + 25	9	11	8	8	22	8	15
MHOM/LK/2002/L60[b]	CL														
MHOM/LK/2002/L59	CL	9	9	11	18	6	13	24 + 25	9	11	7	8	24	8	15
MHOM/IN/1983/CHANDIGARH	VL	10	9	8	22	6	14	18	13	23	8	8	19	8	11
MHOM/IN/2003/LEM4537[c]	CL	10	9	10	22	6	21	21	13	23	8	9	22	8	12
MHOM/IN/2003/LEM4527[c]	CL														
MHOM/KE/1983/NLB 189	PKDL	12	16	9	16	6	16	13	8	11	8	10	30	8	10
MHOM/KE/1985/NLB 323	VL	12	8	9	13	6	16	21	8	11	8	10	30	8	10
IMAR/KE/1962/LRC-L57	Sand fly	11 + 13	16 + 17	8 + 9	12	6	16 + 21	13 + 14	8	11	8	10	30	8	10
MHOM/IL/1998/LRC-L740	VL	10 + 12	11	9	13 + 17	6 + 7	14	6	12 + 15	6 + 12	9	8 + 9	n.d.	23	12

[a,b,c] Paired strains have identical profiles.

closely related to the Sudanese and Ethiopian strains than to the other MON-37 strains. Only few of the smaller nodes in the tree were supported by bootstrap analyses which may reflect the high mutation rates in microsatellites [25], the similarity of *L. donovani* strains or some homoplasy.

The graphical representation of the factorial correspondence analysis (FCA) of the MLMT data (Fig. 2) confirms the assignment of the MON-37 strains to different, clearly separate genetic groups except for those of Sri Lanka and India. It also shows that MON-37 strains of Sri Lanka and India are genetically closer to the strains of the main Indian *Leishmania* population India 1 (IN1) zymodeme MON-2 than to MON-37 strains of other geographic areas. The same is true for the MON-37 strains of Kenya that always group together with Kenya/India-2 (KE/IN2). FCA underlines that strains from Cyprus form a unique genetic group clearly separate from other MON-37 strains.

The Bayesian model-based clustering approach implemented in STRUCTURE grouped the 15 MON-37 strains and the 32 strains of other *L. donovani* included for comparison into 8 genetic clusters (Fig. 3). MON-37 strains were assigned to 5 of these 8 groups: to (1) MON-37 of Israel grouped with the two strains of MON-3 of Iraq and a MON-35 strain of China; (2) MON-37 of Kenya grouped with strains of MON-2, -36 and -38 of Kenya and India (KE/IN2); (3) MON-37 of Cyprus; (4) MON-37 of India and (5) MON-37 of Sri Lanka. The position of the Israeli strain MHOM/IL/1998/LRC-L740 was not clear. It had a mixed membership in different genetic groups at different K values and seemed to be most closely related to the strains from Iraq and China. The strain MHOM/UA/1984/MARZ-KRIM could not be assigned to any of the genetic groups. It was most similar to the MON-37 strains from Cyprus and India. Although the occurrence of gene flow and recombination is increasingly reported for *Leishmania* [15,17,26], these parasites are thought to reproduce predominantly clonally. This contravenes the essential assumption of panmixia in the STRUCTURE algorithm. Nonetheless, the STRUCTURE results corroborate those obtained by genetic distance and FCA. Table 1 lists the assignment of the strains of *L. donovani* to the 8 genetic groups identified by STRUCTURE at $K = 8$.

4. Discussion

Strains of *Leishmania* belonging to the same zymodeme give the impression of constituting a homogeneous group. Of the *L. donovani* strains studied here, those belonging to the zymodeme MON-37 were geographically the most widely spread (Table 1). These two points taken together could lead to the assumption that there is a geographically very widely dispersed group of identical parasites. However, variation among the 14 markers in the microsatellite profiles of the 15 MON-37 *L. donovani* strains revealed that they are genetically diverse and do not belong to one genetic entity (Table 2). Based on their genetic distances they emerge as paraphyletic groups in the phylogenetic tree and were assigned by a Bayesian model-based clustering algorithm to five different

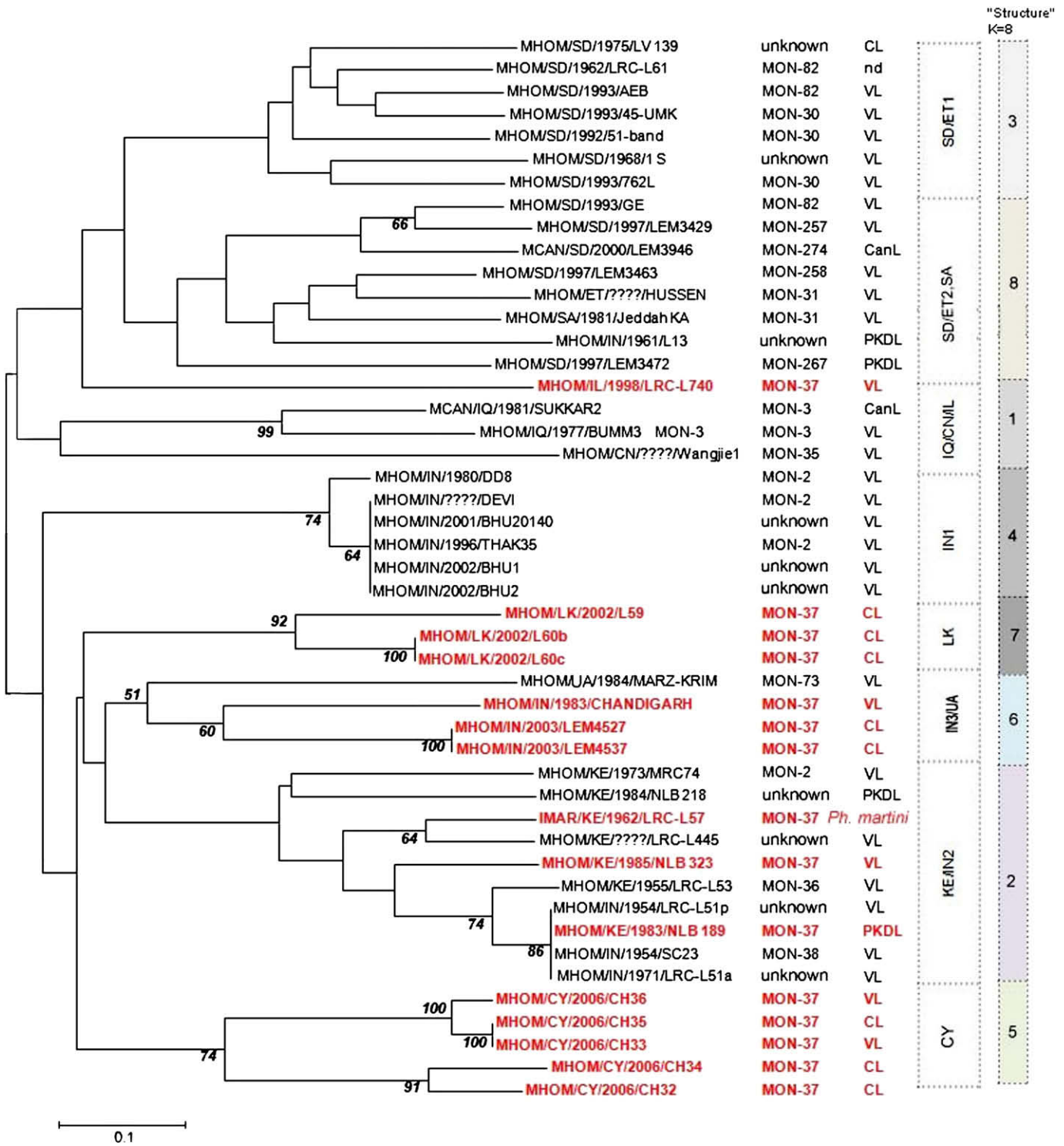

Fig. 1. Midpoint-rooted neighbour-joining tree inferred from Chord distances calculated for the 47 strains of *L. donovani* and the 14 microsatellite markers analyzed. Only bootstrap values >50% are shown.

genetic groups (Fig. 3). These different genetic groups correlate with the geographical origins of the strains. The Kenyan MON-37 strains were indistinguishable from the Kenyan and Indian strains of the MON-36 and MON-38 zymodemes that belong to the KE/IN2 population described by Kuhls et al. [14]. The groups comprising the Sri Lankan strains and the Indian MON-37 strains, one from Himachal Pradesh in northwestern India and the other two from unknown places are related to the main Indian population IN1. The MON-37 strains from these geographical areas are closer to strains of other zymodemes of the same area than to MON-37 strains of different geographical origin. Although the sample sizes for the different areas were limited, the present study represents a solid basis for further investigations deciphering the extent

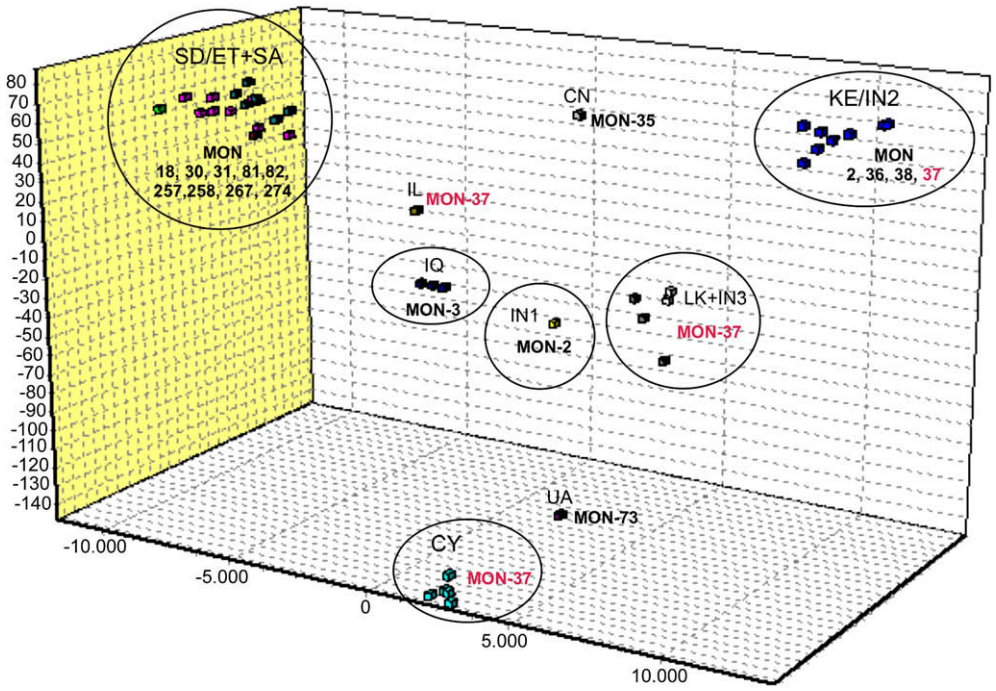

Fig. 2. Factorial correspondence analysis (FCA) of the 47 strains of *L. donovani* studied. The designations SD/ET, IN1, IN3, KE/IN2 are the populations of *L. donovani* defined previously [11,14]. Strains from Cyprus are designated as CY, from Sri Lanka LK, from Iraq IQ, from Israel IL, from Saudi Arabia SA, and from Crimea, Ukraine UA.

of genetic diversity among strains of the MON-37 zymodeme and may help to elucidate their true taxonomic position in the *L. donovani* complex. Future in-depth analysis should include parasites isolated from both mammalian and sand fly hosts.

The Israeli MON-37 strain had an ambiguous position in the tree and in the STRUCTURE analysis, and could not be assigned with any certainty to a particular genetic group. Nevertheless, it appeared to be closer to the strains from Sudan and Ethiopia than to the other MON-37 strains (Fig. 1). This strain was heterozygous at a number of microsatellite loci (Table 2). In addition, when the ribosomal internal spacer was sequenced, different sequence types were detected that resemble sequences from strains of *L. donovani* from the populations Sudan/Ethiopia 1 and 2 [27] and from Iraq (unpublished data). Interestingly, increased numbers of heterozygous loci were also present in three more MON-37 strains, two isolated in Cyprus from human CL cases (MHOM/CY/2006/CH32 and MHOM/CY/2006/CH34) and one isolated in Kenya from a female sand fly of the species *Ph. martini* (IMAR/KE/1962/LRC-L57). Whether these strains were hybrids, aneuploids or simply mixtures of two or more different populations of parasites needs to be clarified by cloning the parasites and sequencing additional genomic targets. The other three MON-37 strains from Cyprus had almost identical microsatellite genotypes. One had caused CL in a Cypriot adult, the other two VL, one affecting a nine months old child of Turkish origin with Epstein Barr virus co-infection [28] and one a 73 years old patient of British origin. All three cases of CL were Cypriots between 45 and 55 years of age. Despite the small number of cases mentioned here, it is suggested that the clinical manifestation of leishmaniasis may relate to the immune status of the host and/or their genetic makeup rather than to differences in the parasite strains.

Antoniou et al. [12] suggested that the MON-37 strains of *L. donovani* recently isolated in Cyprus from human VL and CL cases were originally imported by immigrants from non-European countries. The differences between these Cypriot strains and strains from the Indian subcontinent and Kenya are too substantial to assume very recent introduction by immigrants or infected sand fly vectors. However, since Cyprus is a stepping stone from Africa and Asia to Europe, the possibility of recent introduction of these strains from an endemic area not covered in this study cannot be excluded. On the other hand, if these strains have been circulating in Cyprus for a long time, why was the southern part of the island free of human leishmaniasis with only two reported infantile VL cases since 1935 [29]? Could the lack of clinicians' awareness of leishmaniasis and the unavailability of efficient molecular tools for diagnosis including identification of the parasites account for this?

The above suggests that the epidemiology of leishmaniasis caused by *L. infantum* and *L. donovani* in the Eastern Mediterranean Region needs to be studied more deeply using highly discriminating molecular biological approaches.

The *L. donovani* MON-37 zymodeme encompasses strains with the same enzyme profile phenotype but of different genotype which could be interpreted as contradiction between the two types of analysis. Enzyme analysis has proved efficient in identifying species of *Leishmania* in clinical diagnosis of leishmaniasis and in their epidemiology with regard to identifying sand fly vectors and animal reservoir hosts. Microsatellite analysis has added greater depth to this and

Fig. 3. Assignment of strains of *L. donovani* to different genetic groups by STRUCTURE. This is shown as plots of the estimated membership coefficient which is represented by a single vertical line for each sample. Coloured segments represent a sample's estimated membership in each of the *K*-inferred clusters. Individual isolates can belong to different clusters with membership coefficients summing up to 1 across clusters. The results are shown for *K* (number of clusters) varying from 2 to 8. When $K = 2$, the strains from East Africa, the Indian MHOM/IN/1961/L13, MHOM/IN/1954/SC23 (=LRC-L52), MHOM/IN/1954/LRC-L51a and MHOM/IN/1954/LRC-L51p which are two lines of the same strain and a single strain from Saudi Arabia (Table 1 and Fig. 1) separated from all the other strains of *L. donovani* studied. At $K = 3$, the group of Indian and Kenyan strains including the Kenyan MON-37 strains split away from the Sudanese and Ethiopian strains of *L. donovani*. These two genetic groups remained unchanged at $K = 4$, $K = 5$ and $K = 6$. However, at $K = 7$ the Sudan/Ethiopia/Saudi Arabia group split into the two subgroups SD/ET1 and SD/ET2/SA as described previously [11,14]. At $K = 4$ the Indian and Sri Lankan strains including those belonging to the MON-37 zymodeme separated from those from Cyprus, Israel, Iraq and Ukrainian Crimea. At $K = 6$ strains from Cyprus formed a separate genetic group and MON-37 strains from India and Sri Lanka separated from the Indian MON-2 strains (India 1/IN1). Finally at $K = 8$ the Indian and Sri Lankan MON-37 strains were assigned to different genetic groups. The designations above the multicoloured bars are for populations defined previously [11,14].

makes population genetic studies of the parasites possible. Schonian et al. [30] have reviewed the more discriminating power of the microsatellite analysis in population genetics. Prior to that, Kuhls et al. [14] and Lukes et al. [31] described discrepancies between the results of enzyme analysis and molecular typing, especially regarding the taxonomy of the '*L. donovani*' complex. For East African *L. donovani* strains, molecular techniques have confirmed that *L. donovani* is the only cause of VL in Sudan. *L. infantum* does not appear to exist there and *Leishmania archibaldi* is probably an invalid species definition. In Cyprus, enzyme analysis showed that the human cases of VL and CL were not caused by *L. infantum* as might be suspected but by MON-37 strains of *L. donovani*. Microsatellite analysis revealed that whatever the origin of these *L. donovani* strains, they were not from the places where the other strains and genetic variants of

MON-37 *L. donovani* studied here came from. This demonstrates the different levels of strain characterization by the two types of analyses.

Strains with the same enzyme profile should not necessarily be considered as a genetically monolithic group. Mauricio et al. [26] showed that isoenzymes with identical electrophoretic mobilities had different amino acid sequences, and that the resulting changes in charge and molecular mass could in some cases complement each other so as to bestow the same electrophoretic mobility. Thus, indistinguishable zymodeme phenotypes can be produced by distinct genotypes. Whether this is the case for the enzymes that define the *L. donovani* MON-37 zymodeme needs be clarified.

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