Genomic analysis of natural intra-specific hybrids among Ethiopian isolates of *Leishmania donovani*

James A. Cotton[1*], Caroline Durrant[1], Susanne U. Franssen[1], Tesfaye Gelanew[3], Asrat Hailu[3], David Mateus[4], Mandy J. Sanders[1], Matthew Berriman[1], Petr Volf[2], Michael A. Miles[4], Matthew Yeo[4*]

[1]Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, United Kingdom.

[2]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic.

[3]Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia

[4]Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom

*authors for correspondence. JAC: james.cotton@sanger.ac.uk; MY: Matthew.Yeo@lshtm.ac.uk.

Abstract

Parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) cause widespread and devastating human diseases, ranging from self-healing but disfiguring cutaneous lesions to destructive mucocutaneous presentations or usually fatal visceral disease. Visceral leishmaniasis due to *Leishmania donovani* is endemic in Ethiopia where it has also been responsible for major epidemics. The presence of hybrid genotypes has been widely reported in surveys of natural populations, genetic variation reported in a number of *Leishmania* species, and the extant capacity for genetic exchange demonstrated in laboratory experiments. However, patterns of

recombination and evolutionary history of admixture that produced these hybrid populations remain unclear, as most of the relevant literature examines only a limited number (typically fewer than 10) genetic loci. Here, we use whole-genome sequence data to investigate Ethiopian *L. donovani* isolates previously characterised as hybrids by microsatellite and multi-locus sequencing. To date there is only one previous study on a natural population of *Leishmania* hybrids, based on whole-genome sequence. The current findings demonstrate important differences. We propose hybrids originate from recombination between two different lineages of Ethiopian *L. donovani* occurring in the same region. Patterns of inheritance are more complex than previously reported with multiple, apparently independent, origins from similar parents that include backcrossing with parental types. Analysis indicates that hybrids are representative of at least three different histories. Furthermore, isolates were highly polysomic at the level of chromosomes with startling differences between parasites recovered from a recrudescent infection from a previously treated individual. The results demonstrate that recombination is a significant feature of natural populations and contributes to the growing body of evidence describing how recombination, and gene flow, shape natural populations of *Leishmania*.

Author Summary

Leishmaniasis is a spectrum of diseases caused by the protozoan parasite *Leishmania*. It is transmitted by sandfly insect vectors and is responsible for an enormous burden of human suffering. In this manuscript we examine *Leishmania* isolates from Ethiopia that cause the most serious form of the disease, namely visceral leishmaniasis, which is usually fatal without treatment. Historically the general view was that such parasites reproduce clonally, so that their progeny are genetically identical to the founding cells. This view has changed over time and it is increasingly clear that recombination between genetically different *Leishmania* parasites occurs. The implication is that new biological traits such as virulence, resistance to drug treatments or the

53 ability to infect new species of sandfly could emerge. The frequency and underlying mechanism
54 of such recombination in natural isolates is poorly understood. Here we perform a detailed whole
55 genome analysis on a cohort of hybrid isolates from Ethiopia together with their potential parents
56 to assess the genetic nature of hybrids in more detail. Results reveal a complex pattern of mating
57 and inbreeding indicative of multiple mating events that has likely shaped the epidemiology of the
58 disease agent. We also show that some hybrids have very different relative amounts of DNA
59 (polysomy) the implications of which are discussed. Together the results contribute to a fuller
60 understanding of the nature of genetic recombination in natural populations of *Leishmania*.
61
62 **Introduction**
63
64 *Leishmania* is a diverse genus of kinetoplastid protozoan parasites from the family
65 trypanosomatidae. These parasites are best known as the cause of human and animal
66 leishmaniasis, which is a clinically important neglected tropical disease affecting millions of people
67 and causing a tremendous burden of mortality and morbidity (Herricks et al. 2017; Alvar et al.
68 2012). Leishmaniasis comprises a spectrum of related diseases which, depending on the species,
69 results in various presentations ranging from small, self-healing cutaneous lesions to widespread
70 disseminated lesions, destructive mucosal and mucocutaneous pathology, and visceral disease
71 that is usually fatal in the absence of effective chemotherapy (Herwaldt 1999). *Leishmania* have
72 a digenetic (two host) life cycle involving a vertebrate host and 166 different species of
73 phlebotomine sand fly that have been implicated as vectors (Akhoundi et al. 2016), although
74 alternative invertebrate vectors may exist for some species (Seblova et al. 2015). Vertebrate hosts
75 encompass a wide range of mammals or reptiles, and around 20 species of *Leishmania* have
76 been reported to infect humans (Akhoundi et al. 2016).
77

Historically, the population structure of *Leishmania*, other trypanosomatids and indeed most protozoan parasites was considered to be largely clonal (Tibayrenc, Kjellberg, and Ayala 1990): the presumption was that admixture between members of the same clone, or between very closely related parasites was absent or rare, with minimal impact on population structure. However, at the time the clonal theory was first proposed, most population genetic data for trypanosomatids was based on inadequate sampling and use of low-resolution markers unlikely to detect admixture between genetic groups (Ramírez and Llewellyn 2014). Subsequently, extensive work using multilocus sequence typing and microsatellite markers has produced a foundation for understanding of the population genetics of some *Leishmania* species (Akhoundi et al. 2017; G. Schönian, Kuhls, and Mauricio 2011; Gabriele Schönian, Cupolillo, and Mauricio 2012; Ramírez and Llewellyn 2014). Most natural *Leishmania* isolates have surprisingly little heterozygosity, which has been widely ascribed to extensive selfing (Rougeron et al. 2009, 2010; Kuhls et al. 2007), although aneuploidy variation could also contribute (Sterkers et al. 2014). In contrast there have also been a number of reports of heterozygous natural isolates possessing a mixture of alleles associated with different populations (Schwenkenbecher et al. 2006; Kuhls et al. 2013; Gelanew et al. 2014) and even different species (Ravel et al. 2006; Hamad et al. 2011), suggesting a hybrid origin of these isolates. There is therefore a growing body of evidence for genetic exchange between natural populations of several *Leishmania* species.

Laboratory genetic crosses between at least two *Leishmania* species have been achieved in the sand fly vectors (Akopyants et al. 2009; Sadlova et al. 2011), and viable hybrids have been achieved between geographically disparate sources of *L. major* (Inbar et al. 2013). Here many hybrids possess genotypes consistent with classical meiosis; however, aneuploidy with recurrent triploidy and loss of heterozygosity (LOH) were also observed. Interspecific *L. major/L. infantum* crosses have also been performed with segregation of cutaneous and visceral traits (Romano et al. 2014). However, distinct male or female gametes of *Leishmania* have not been described,

104 although haploid stages of *Trypanosoma brucei* have recently been discovered in tsetse flies
105 (Peacock et al. 2014).
106
107 The fact that *Leishmania* can undergo genetic exchange is of profound epidemiological
108 importance. Genetic exchange may facilitate adaptation to new vectors, mammalian hosts or
109 other ecological niches. For example, *Leishmania infantum/major* hybrids infect *Phlebotomus*
110 *papatasi*, a non-permissive vector for *L. infantum* that is widespread in the Indian subcontinent
111 (Volf et al. 2007). Hybrids between *L. braziliensis* and *L. peruviana* have also been implicated as
112 agents of destructive forms of mucocutaneous leishmaniasis (Nolder et al. 2007). Genetic
113 exchange could also lead to the spread between populations of genes associated with resistance
114 to drugs. Reassortment can potentially affect sensitivity and specificity of diagnostic methods and
115 hybrid vigour (heterosis) could also affect virulence or transmission potential. Such implications
116 are particularly worrying in the context of recombination contributing to the generation of novel
117 visceralising traits in populations previously causing only dermal symptoms, or if adaptation to
118 new vector species allows existing visceralising parasites to become more widespread.
119
120 Genome-wide sequence data are crucial to explore fully the extent of hybridisation and to identify
121 the mechanisms by which hybrids are formed (Twyford and Ennos 2011). Data from only a few
122 genetic loci may be adequate for identifying hybrids if admixture is recent or the populations have
123 not extensively interbred. However, sparse markers are less sensitive in identifying complex,
124 infrequent or ancient admixture, where only a small fraction of the genome may derive from any
125 one parent. These kinds of events are known to occur in microbial eukaryote pathogens (Ropars
126 et al. 2018; McMullan et al. 2015; Desjardins et al. 2017). Being able to describe signatures of
127 genetic exchange in detail is important as other processes can explain hybrid patterns of
128 genotypes; for instance, parasexual processes based on fusion of cells followed by mitotic
129 crossing-over have previously been observed in some protozoa and are well-described in fungi

(Ene and Bennett 2014). Parasexual recombination can also produce similar inheritance patterns and some evidence of this is seen in an experimental cross between different *Leishmania* species (Romano et al. 2014), where many progeny are also highly aneuploid. Whole-genome sequence data have been reported for only a single population of hybrid *Leishmania* from Turkey (Rogers et al. 2014). This population appears to have originated from a single hybridisation event between genetically disparate lineages within the *L. donovani* species complex. One of the parents appeared to be an *L. infantum*, but the precise parentage of this population remains unclear as no parental genotypes were isolated in the same region. While genomic patterns were consistent with meiosis they do not formally exclude the possibility of a parasexual process.

Here we present a detailed genomic analysis of a natural hybrid population of *L. donovani* originating from Ethiopia. East African strains of *L. donovani* are particularly diverse, consisting of two main populations: one comprising strains from northern Ethiopia and Sudan, the other strains from southern Ethiopia and Kenya (Zackay et al. 2018) and correspond to the areas populated by two different major sand fly vectors – *Phlebotomus orientalis* being the main vector in northern Ethiopia and Sudan and *Phlebotomus martini* in the South – although other vectors have also been implicated (Seblova et al. 2013). These two geographically (and genetically) isolated populations of *L. donovani* in Ethiopia also differ in clinical phenotypes (Gelanew et al. 2010). High inbreeding, seemingly incompatible with strict clonality, was observed in strains from northern Ethiopia. Microsatellite (Gelanew et al. 2010) and MLST markers (Gelanew et al. 2014) have confirmed the presence of sympatric putative parental genotypes and hybrid progeny genotypes of *L. donovani* in isolates from the northern population. Here, we apply whole-genome sequencing data to characterise more fully these Ethiopian *L. donovani* to confirm that isolates are true hybrids that originate from recombination between two different sympatric lineages. We reveal a complex pattern of inheritance implying multiple independent origins from similar parents,

and backcrossing with parental types. Extensive polysomy, at the level of chromosomes, is apparent in some hybrids, the significance of which is discussed.

Results

Genome sequencing

From each of 11 putative hybrid isolates, 1,600-2,300 Mb of sequence data (Illumina 100 bp paired-end reads) (Table 1) were generated. When mapped against the reference genome for Ethiopian *L. donovani* (Isolate LV9, WHO code: MHOM/ET/67/HU3, Rogers et al. 2011) these data produced at least 40-fold median coverage across the isolates. Generally, coverage was consistent across the genome, with more than 30 reads per sample covering at least 90% of the genome (Table 1). SNP calling identified an average of 75,775 SNPs between each individual isolate and the reference genome, and this was relatively consistent across the panel, varying between 63,042 and 89,636 (Table 1). In contrast to this consistency in the number of variable sites, the proportion inferred to be heterozygous varied considerably. Some isolates showed very low heterozygosity (for example LdM256, 0.015) while for others almost half of variant sites were inferred to be heterozygous (for example LdDM62, 0.468). Most isolates exhibiting low levels of heterozygosity (<0.1) were previously identified as putative parental genotypes (Gelanew et al. 2014), in contrast putative hybrids showed much higher levels (>0.3); with the exception of one putative parental type (LdDM481) with heterozygosity more similar to that of parental isolates (0.267). In terms of large (>100 bp) structural variants, we observed 368 deletions, 282 inversions, 169 duplications and 264 translocations. However, many of these variants do not segregate among the recent Ethiopian isolates sequenced here (see Figure S1), with most being heterozygous in all or most of these isolates (Table S1, Figure S1). A single 18 bp homozygous insertion on chromosome Ld33 was present in all of the Ethiopian isolates sequenced here, which was also present in reference strains LV9 and JPCM5 but not present in BPK282.

Table 1: Sequencing data and summary statistics of read mapping and variant calls

isolate	WHO name	Previous classification (Gelanew et al, 2014)	Sequence reads (total Mb)	Median mapped coverage	Proportion of genome with >= 30x coverage	# of variable sites against LV9	Hetero-zygosity
LdDM19	MHOM/ET/2007/DM19	Possible hybrid*	21383530 (2138.3)	53	0.97	80930	0.301
LdDM20	MHOM/ET/2007/DM20	parental type A	19098154 (1909.8)	49	0.96	74749	0.024
LdDM62	MHOM/ET/2007/DM62	hybrid	19577434 (1957.7)	45	0.94	89636	0.468
LdDM256	MHOM/ET/2008/DM256	parental type B	19854188 (1985.4)	51	0.96	63042	0.015
LdDM257	MHOM/ET/2008/DM257	parental type B	23066268 (2306.6)	59	0.97	63088	0.016
LdDM259	MHOM/ET/2008/DM259	parental type B	16023002 (1602.3)	42	0.91	63209	0.028
LdDM295	MHOM/ET/2008/DM295	hybrid	20010052 (2001.0)	51	0.96	87546	0.388
LdDM297	MHOM/ET/2008/DM297	parental type A	20651456 (2065.1)	52	0.96	76530	0.072
LdDM299	MHOM/ET/2008/DM299	hybrid	22442162 (2244.2)	53	0.97	88605	0.462
LdDM481	MHOM/ET/2009/DM481	parental type B	20625354 (2062.5)	53	0.97	82728	0.267
LdDM559	MHOM/ET/2009/DM559	parental type B	18753496 (1875.3)	51	0.96	63469	0.030

186 **Genome-wide variation identifies distinct subgroups with different levels of**
187 **heterozygosity**
188
189 A phylogenetic tree and principal components analysis of the SNP data suggesting that these 11
190 isolates are divided into multiple groups, with two parental groups and at least two putative hybrid
191 groups (Figure 1). As expected the two parental groups are composed of isolates with low
192 heterozygosity. The first parental group, comprises LdDM259, LdDM559, LdDM257, LdDM256
193 and the second, comprises LdDM20, LdDM297. LdDM481 is an outlier in that it is a highly
194 heterozygous sample that forms a distinct lineage, intermediate between the two parental groups
195 but clearly distant from the other four putative hybrid isolates (LdDM19, LdDM62, LdDM295,
196 LdDM299). Two isolates (LdDM62 and LdDM299) isolated from the same patient (a post
197 treatment recrudescence in an HIV patient) appear very similar on the tree and PCA. The first
198 two principal components (PCs; Figure 1b) explain 86.32% of the variance (60.32% and 20%
199 respectively) in the data broadly reflecting previous interpretation of these isolates as hybrids and
200 parental genotypes, the putative hybrid isolates being intermediate between the sets of parentals.
201 An interesting exception is LdDM481, which appears distinct from all other samples regarding the
202 first PC, with all subsequent PCs showing similar patterns, up to PC5, where LdDM19 appears
203 as distinct from the other isolates: however, this axis encompasses only 1.9% of the total variation
204 in these data. In the phylogeny, inclusion of three additional reference genomes (LV9, an *L.*
205 *donovani* isolate from an Ethiopian VL patient; JPCM5, a Spanish canine *L. infantum*, and
206 BPK282 from a Nepalese VL case) revealed the diversity present in this Ethiopian cohort and
207 their distant relationship to both *L. donovani* in the Indian subcontinent and *L. infantum* (Figure
208 1a). The reference isolate LV9, originally isolated in 1967, appears to be closely related to one of
209 the parental populations.
210

Chromosome copy number for each isolate was inferred from read depth and allele frequencies at heterozygous sites (see methods, Figure 2). Broadly, chromosomes across the majority of isolates were inferred to be diploid. The exception is chromosome 31, which, as usual in *Leishmania*, was inferred to be highly polysomic with at least four copies present in all isolates. Chromosomes 5, 6, 8, 20 and 35 were also observed at higher dosage, being at least trisomic in 6 of the 11 isolates. Two samples stood out as being more highly polysomic than others: LdDM19 was inferred to be tetrasomic at three chromosomes (13, 31 and 3), while LdDM299 was strikingly polysomic. For this isolate, allele frequency data suggested a minimum of tetrasomy across chromosomes, with half the chromosomes inferred at even higher dosage (6 pentasomic, 9 hexasomic, 1 heptasomic, with chromosomes 31 and 33 octasomic).

The high somy of LdDM299 is of particular interest given that LdDM62, the pre-treatment sample from the same HIV infected patient, has somy similar to the other hybrid isolates. These two isolates are otherwise genetically very similar, differing at only 4,484 sites across the genome (LdDM62 differs from the other hybrid isolates at 38,023 and 25,765 sites). The difference in allele frequency distribution between LdDM62 and LdDM299 is clear: for example, chromosomes 11, 12 and 33 all show a clear peak in allele frequencies close to 0.5 in LdDM62 suggesting disomy (or at least an even chromosome dosage), but peaks at 0.33 and 0.67 in LdDM299, suggest a higher dosage of at least 3 copies (Figure 2b). The very high somy of other chromosomes is then inferred from the ratio of coverage (Figure 2a). We attempted to confirm the high ploidy of LdDM299 using flow cytometry to measure DNA content. Cells from the same population of cells that was sequenced were not available, but a cloned population separated from the sequenced cells by more than 8 *in vitro* passages was analysed. DNA content of these cells was suggestive of diploidy (S1 Fig), leaving some uncertainty about the precise somy of the LdDM299 isolate. During SNP-calling, the copy number of individual chromosomes is specified. We thus confirmed that our main results are insensitive to the assumed somy of the isolates by repeating most

237 analyses with genotypes called as though all isolates are diploid: in all cases the conclusions from
238 our analyses are qualitatively the same with diploid genotypes, or genotypes called using the
239 inferred somies.
240
241 **Patterns of inheritance from putative parental populations**
242
243 Variants at many sites were shared by putatively hybrid isolates and either one or other of the
244 parental group; with relatively few variants present only in the hybrids and not found elsewhere
245 (Figure 3). LdDM481 was an exception, possessing a moderately high number of private variants
246 and also sharing some different variants with the parental groups compared to other hybrids,
247 particularly with parent B where other hybrid isolates shared substantially more variation.
248
249 To understand further the origins of the hybrid parasites, we identified SNP variants that are fixed
250 differences between the two groups of parental parasites (excluding LdDM481) and used these
251 as markers to identify the likely origin of variants identified in the hybrids, effectively 'painting' the
252 hybrid isolate chromosomes by their likely ancestry under the hypothesis that these isolates
253 originated as hybrids between the parental groups or close relatives. We identified a total of
254 49,835 such 'parent-distinguishing SNP' sites at which the two parental populations were
255 completely fixed for different alleles. These sites are distributed across all chromosomes. For the
256 vast majority of sites, across all putative hybrid isolates, genotypes consisted of a combination
257 these parental alleles (Table 2), supporting the notion that these isolates originated as hybrids
258 between two parental types. The different categories of sites are strikingly unevenly distributed
259 across the genomes suggestive of genome wide hybridisation (Figure 4). For some chromosomes
260 these SNP markers appear to be uniformly inherited from a single parent or show uniform
261 heterozygosity with one allele from each parental type. Most chromosomes, however, show
262 multiple blocks of sequential SNPs of different origins, revealing a patchwork of blocks of different

ancestries. Isolate LdDM481 is an exception: the “blocky” structure visible in other isolates is not apparent, there are less than half the number of heterozygous sites of shared origin in comparison to any other hybrid isolates, and five times the number of genotypes containing alleles other than those fixed in the parental groups, although this still represented only 54 sites.

Table 2 - Distributions of parental-distinguishing SNPs in putative hybrid isolates. Values are numbers of sites in each isolate from each category. For polysomic chromosomes, sites with at least one allele from each parent are grouped as ‘heterozygous AB’ irrespective of dosage of A and B alleles.

isolate	Homozygous parent A	Homozygous parent B	Heterozygous AB	Genotype not unambiguously inferred from reads	Other genotype
LdDM19	21,171	10,419	16,633	1,602	10
LdDM62	15,600	2,103	29,779	2,343	10
LdDM295	25,482	1,429	21,032	1,880	12
LdDM299	15,868	2,036	18,571	13,352	8
LdDM481	31,104	10,271	7,400	1,006	54

To get a more quantitative understanding of the distribution of these parent distinguishing SNP sites in blocks inherited from each parent we developed a hidden Markov model (HMM) to assign putative ancestry for sites without parent-distinguishing SNPs. The HMM assigns every 100 bp window of the genome to three states: either homozygous 'parent A', homozygous 'parent B' or heterozygous (Figure 5a). The advantage of the HMM is that it can statistically assign windows even in the absence of any parent-distinguishing variants (or where those variants are ambiguous), under the assumption that transitions between the states occur in a regular way across the genome if there is no direct evidence of a change. The HMM assigns significantly different proportions of the genome to each category for different isolates (Figure 5a), and the uneven distribution of sites in each category across the genome is reflected in the fact that these proportions are quite different from the proportion of parent-distinguishing SNP sites assigned to each category. This model also estimates the length of 'runs' that form blocks of genome with a single inheritance pattern. The distribution of these block lengths provides information on the relative age of hybrids, as we would expect recombination to have broken down blocks from older introgression events more than recent events. Longer blocks suggest that fewer hybridisation events have occurred between admixed clones but backcrossing to parental clones or related parasites would also contribute to longer blocks. The block length distribution varies between isolates (Figure 5b): it suggests that LdDM62 and LdDM299 represent a more recent hybridisation with a 'parent B' type than any 'parent A' type, and that LdDM295 may originated from a more recent hybrid (or with fewer hybridisations) between the two parental types than LdDM19. For at least two isolates (LdDM62 and LdDM299) the inheritance is asymmetrical, in that they are inferred to have inherited different proportions of their genome from each of the parental types, suggesting that these isolates did not originate by crossing within a 'founder' hybrid population, but involved some degree of backcrossing with parent B types.

Reconstructing haplotypes confirms that variants of each parental type are linked

We used reads and read pairs to phase locally heterozygous sites that were physically close to each other in the genome into sets of variants known to be present on a single haplotype. We subsequently identified regions at which all heterozygous positions were phased in all 15 isolates, so that we have unambiguous information about the haplotypes present in these regions. We inferred haplotype phylogenies for nine such regions that were at least 3 kb long and had an average of at least 4 heterozygous sites per isolate; this included 9 of the 16 'fully phased' regions of 3 kb or longer (figure 6). All 9 blocks were on different chromosomes.

In most blocks (7 out of 9; Figure 6a-e, g, h) the two haplotypes for each of the four hybrid isolates (LdDM19, LdDM62, LdDM295 and LdDM299) cluster in different parts of the phylogeny. For 6 of these (Figure 6a-d, g, h), phylogenies show the expected pattern if the hybrid isolates originated from a simple, single hybridisation between the two parental types: a long branch of the haplotype tree separates all parent A haplotypes together with one haplotype of each of the 4 hybrids from parent B haplotypes with the second haplotype of each hybrid. In one block (Figure 6e) on chromosome 17 the two haplotypes for each hybrid isolate divide into two clusters as expected, but the two 'parent A' isolates (LdDM20 and LdDM297) appear in different clusters. In the chromosome 34 block, both haplotypes for one hybrid isolate (LdDM295) clustered with the same parental group – the parent A isolates (figure 6i). All of these haplotypes are consistent with a hybrid origin for the isolates but with a more complex history than a simple, single hybridisation between the two parental populations. This could involve further recombination either by 'intercrossing' within the hybrid population or backcrossing to the parental types.

Only the final block (Figure 6f) does not support a simple hybrid origin for these isolates, as the two haplotypes for each isolate cluster together. Further rounds of crossing, with a different history

for LdDM19 and the other hybrid isolate, would explain the pattern at this locus. Examining the alignments for these blocks did not reveal any sign of recombination within reconstructed haplotypes, but did reveal some haplotypes in the putative hybrids that differ from either of the putative parental types - for example at the haplotype block on Ld10 all of the hybrids share one haplotype that is very similar to those in *L. infantum* JPCM5 but missing from any of the other *L. donovani* isolates (Figure 6b; figure 7a). Either the parent B isolates are a poor proxy for the true parental types at this locus, or the history of the hybrid isolates includes crossing with more than two parental populations.

The pattern for LdDM481 haplotypes was more complex: in 7 out of 9 trees the two haplotypes for this isolate appear in the same cluster: twice with parent B haplotypes (figure 6g,h) although never very closely related to these; four times with parent A haplotypes (figure 6b,d,f,i). In one other case (Figure 6e) the parent A isolates are themselves non-monophyletic. At many of these loci, LdDM481 has haplotypes not present in other isolates. At two other loci (on Ld05 and Ld12; Figure 6a, c) LdDM481 is heterozygous for one haplotype not observed elsewhere and for one haplotype shared with parent A isolates and the hybrids. At the phased locus on Ld32 (Figure 6h), the LdDM481 haplotype is apparently distantly related to BPK282; although closer inspection of this locus shows they are united by their lack of alleles present in other isolates rather than shared characters (Figure 7b).

For the other “outgroup” isolates of *L. infantum* and *L. donovani*, the two haplotypes from each isolate consistently cluster together. Wherever parent A haplotypes are monophyletic, the Ethiopian LV9 isolate haplotypes group with them. Nepalese *L. donovani* BPK282 tends to group with the parent B isolates but is often clearly distinguishable from them; the position of *L. infantum* JPCM5 haplotypes on these trees is more variable.

353 **Kinetoplast (kDNA) phylogeny**

354

355 The kDNA maxicircle is homologous to the mitochondrial genome of other eukaryotic groups
356 (Jensen and Englund 2012), and is thought to be uniparentally inherited in *Leishmania* (Akopyants
357 et al. 2009) and trypanosomes (Turner et al. 1995). The maxicircle phylogeny (Figure 8) shows a
358 close relationship between the hybrids, parental type A, LdDM481, and the historical reference
359 LV9 isolate, and are phylogenetically distinct from parental B isolates. This contrasts with the
360 nuclear phylogeny, which shows the hybrid samples as somewhat more closely related to parent
361 A isolates but clearly intermediate between both parental groups: here, the parental A isolates do
362 not even form a monophyletic group, with LV9 and hybrid LdDM297 clustering with one parental
363 type. Surprisingly, the mitochondrial phylogeny suggests some divergence between LdDM299
364 and LdDM62, despite them originating pre and post treatment from the same patient. However,
365 supporting bootstrap values were low for all relationships in this part of the tree. The outlier isolate
366 LdDM481 also appears less distant from the cluster comprising parental group A and hybrids than
367 indicated from nuclear SNP variation, even though mutation rates are likely to be an order of
368 magnitude greater for mitochondrial data than for the nuclear genome. The uncertainty in the
369 precise relationships between isolates notwithstanding, the close relation between parental group
370 A and the hybrid isolates suggests the hybrids uniparentally inherited parent type A mitochondrial
371 genomes, and gives some support to the idea that these all originated from a single initial cross,
372 although these data cannot exclude that there is some bias in the inheritance of kDNA between
373 strains, or that this shared kDNA type reflect subsequent backcrossing rather than the original
374 hybridisation.

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Discussion

Hybridisation in *Leishmania* has now been demonstrated experimentally and is also observed in natural isolates across different species. These include multiple *L. braziliensis-L. peruviana* hybrids in Peru (Nolder et al. 2007), *L. infantum-L. major* hybrids (Volf et al. 2007) and a widespread lineage of *L. tropica* that appears to be disseminated from a recent hybridization event (Schwenkenbecher et al. 2006). However, the only previous whole genome analysis of hybrid *Leishmania* isolates identified a population from Turkey that appeared to be hybrids between a MON-1 genotype type of *L. infantum* and another member of the *L. donovani* species complex (Rogers et al. 2014). In that case, isolates appear to be generated solely from continued crossing within an initial hybrid population without back-crossing to either parental type. The genetic heritage of Ethiopian hybrids we describe here must be more complex. More specifically we propose that these isolates must have originated from more than one crossing event between similar parents. Additionally, crosses must have occurred between either different hybrids types or between hybrids and parentals subsequent to the 'founding' outcrossing event.

In more detail, our results are indicative of complex *L. donovani* populations in northern Ethiopia, with the relatively small number of isolates sequenced here representing at least three different histories. We find two distinct parental groups of isolates with low heterozygosities. The first parental group, comprising LdDM259, LdDM559, LdDM257, LdDM256 and the second, comprising LdDM20, LdDM297. We confirm that four of the heterozygous isolates (LdDM19, LdDM62, LdDM295 and LDM299) possess hybrid genotypes that are phylogenetically intermediate between these two parental groups. Most blocks of variants in these genomes can be interpreted as being inherited from one, or both of the parental populations. The large-scale structure of these genomes as blocks of variants of particular ancestry suggests that these are relatively recent events, at least in terms of the number of crossing-over events that have occurred

since. There are however substantial differences between the hybrids in that the number of variants shared by individual hybrid isolates and the parentals, and also their distribution across the genomes differs substantially.

Results are indicative of at least three different histories of crossing between parental types, and probably within the hybrid population itself, with LdDM299 and LdDM62 showing very similar patterns, while both LdDM19 and LdDM295 are distinct. Using a hidden Markov model, we estimated the lengths of blocks of sequence inferred to originate from each parental population. The distribution of sizes of contiguous sequence fragments derived from each parent was not consistent between the hybrids, probably reflecting differences in the timing of hybridization events; we expect that continued interbreeding and the accompanying crossing-over gradually broke down these blocks of ancestry. Hence, blocks inherited from parents in older crossing events have smaller stretches of continuity. Our HMM results thus gives qualitative insight into the relative age of the different events that gave rise to these hybrid isolates. Using this approach we infer that LdDM62 and LdDM299 have crossed onto a parent B like ancestor more recently than to parent A, while the ancestry of LdDM19 and LdDM295 from both parents is approximately equally old. This approach assessing length of inherited haplotypes has been previously used to gain quantitative results into the history of human populations (Pugach et al. 2011), but parameterising this kind of approach in *Leishmania* seems challenging, given the facultative nature of sexuality in this genus, and our current lack of understanding of recombination in *Leishmania*. In particular, we do not quantitative information on the mutation rate, recombination rate or even likely generation time of *Leishmania* in vivo. In all cases a greater proportion of the genomic variation was shared with the ‘parent A’ population, suggesting a more recent common ancestry with this population or backcrossing with this population during evolutionary history. The close relatedness between the LV9 isolate, isolated in the Humera district of Ethiopia in 1967, and one of the parental groups indicates that parental like genotypes have been present in this

region for extended periods of time. While the age of the hybridisation events we have described are unclear, the presence of genotypically stable "parental donors" over time may have facilitated the emergence of multiple hybrid populations. In summary, while we cannot reconstruct the precise history of this population, our data confirm that the population of *L. donovani* in Ethiopia has undergone multiple rounds of hybridisation, including more complex patterns of crossing than simple F1 hybridisation between parents or subsequent crossing within a hybrid population.

The highly heterozygous isolate LdDM481 emerged as a consistent outlier that forms a distinct lineage, intermediate between the two parental groups but also distinct from the other four putative hybrid isolates. The presence of haplotypes at a number of loci in this isolate which are not present in either parental population makes it seem unlikely that this is a very recent hybrid between the two parental groups in the current cohort. One explanation is that DM481 is simply a representative of a distinct, divergent population, which appears plausible considering the genetic diversity within *L. donovani* that has previously been identified within this region (Gelanew et al. 2010, 2014; Zackay et al. 2018). A more unlikely scenario is that DM481 has some recent common ancestry with the parent A population but less so with population B.

The multiplicity of hybrid and parental genotypes within this small sample of isolates from northern Ethiopia suggests that genetic exchange is commonplace among *L. donovani* populations transmitted by *P. orientalis,* and that resulting hybrid progeny may be widely disseminated. This complex evolution also implies that co-infection of *P. orientalis* with different *L. donovani* isolates occurs frequently, at least in this region. It is currently unknown if hybrids are most likely to emerge when a sand fly is co-infected with different *Leishmania* strains ingested in the same blood meal or subsequent feeds. For *T. brucei* there is evidence that the production of hybrid genotypes is most successful when both parental types are taken up in a single meal by the tsetse vector (Peacock, Bailey, and Gibson 2016).

457

458 The pattern of polysomy we observe across the cohort did not reflect the phylogenetic relationship
459 between isolates or their assignment to hybrid or parental classes; this is consistent with a highly
460 dynamic chromosome complement in *Leishmania* promastigotes described both experimentally
461 (Sterkers et al. 2011) and in field samples, for example in *L. donovani* in the Indian subcontinent
462 (ISC; Imamura et al., 2016). Indeed, aneuploidy patterns do not seem to strongly segregate
463 between Ethiopian *L. donovani* populations (Zackay et al. 2018) despite the degree of nucleotide
464 diversity identified in these samples from one region of Ethiopia alone being much higher than in
465 the ISC. For example, the average pair of samples in the main ISC population differ at only 88.3
466 sites whereas in the current cohort even two of the closely related 'parent B' isolates vary at an
467 average of 1038 sites. Aneuploidy is known to be beneficial in allowing some single celled
468 eukaryotes, for example *Saccharomyces* and *Candida*, to rapidly generate adaptive diversity (A.
469 M. Selmecki et al. 2015), and likely contributes to adaptation in *Leishmania* (Mannaert et al. 2012;
470 Prieto Barja et al. 2017). Aneuploidy is known to impact gene expression in *Leishmania*
471 promastigotes (Dumetz et al. 2017; Iantorno et al. 2017). As *Leishmania* lacks classical regulation
472 of transcription at initiation through promoters, this could contribute to parasite adaptation to at
473 least some conditions (Laffitte et al. 2016; Mannaert et al. 2012). However, it is unclear how
474 extensive aneuploidy variation in cultured promastigotes is relevant to the situation in either
475 natural vectors, or in amastigotes *in vitro* or *in vivo*: while it is clear at least some variation does
476 occur in both (Dumetz et al. 2017; Kumar et al. 2013) it appears to be much less widespread than
477 in *in vitro* culture.

478

479 A striking result is that the sequence data suggests that isolate LdDM299, a recrudescent infection
480 (from LdDM62) taken from the same patient was remarkably polysomic across all chromosomes
481 relative to other isolates. Previous flow cytometry measurements of DNA content were suggestive
482 of diploidy for both strains LdDM62 and LdDM299 and all other strains in the cohort (Gelanew et

al. 2014) and are therefore incongruent. However a potential confounder was that the original cloned line that was sequenced was not available for cytometric analysis, with the isolate having undergone additional passages (>8). Somy in *Leishmania* can vary dramatically and rapidly in culture (Lachaud et al. 2014; Dumetz et al. 2017) and prolonged *in vitro* culture is known to systematically reduce ploidy in experimentally derived *T. cruzi* (Lewis et al. 2009). Here, relative somy between chromosomes is inferred from the coverage depth of reads mapped to each chromosome, while the baseline somy is determined from the allele frequency distribution. In principle, this could be misleading if the samples sequenced were mixtures of clones with many different somy levels, and single cell approaches such as FISH or single cell sequencing would be needed to fully disentangle this (Dujardin et al. 2014). However, the differences in allele frequency distributions between LdDM62 and LdDM299 for many chromosomes is particularly striking (Figure 2b), so there are at least genuine differences in the complement of chromosomes between the sequenced isolates. The consistently high dosage of some chromosomes – most strikingly chromosome 31 – are also broadly consistent with previous reports (Rogers et al. 2011; Downing et al. 2011; Dumetz et al. 2017). Together these provide reassurance that somy inferences are correct. We speculate that the apparent remarkable differences in somy between LdDM62 and LdDM299 isolated from the same HIV patient, could be an adaptive response to either chemotherapy or suppression of the patient's immune response. SNP differences between LdM62 and LdM299 isolates were minimal, so aneuploidy variation could be a convenient mechanism to alter gene expression in response to drug pressure, as demonstrated in *Leishmania* (Mannaert et al. 2012) and conclusively in resistance of some pathogenic fungi to azole drugs (Kwon-Chung and Chang 2012; A. Selmecki, Forche, and Berman 2006).

Broadly, mitochondrial phylogenies corresponded to the expected nuclear genotypes (Figures 1 and 8 respectively). These data suggest hybrids uniparentally inherited parent type A mitochondrial genomes in agreement with inheritance patterns seen previously in other

509 trypanosomatids (Messenger et al. 2012); (Satoskar and Snider 2009). There was some
510 indication that LdDM62 and LdDM299, isolated from the same patient pre and post treatment,
511 possessed some mitochondrial sequence diversity. However, bootstrap support was low. While
512 all analyses support the clustering of LV9, parent A and hybrid isolates, the precise placement of
513 different isolates within this cluster varied with details of the mitochondrial maxicircle assembly
514 approach. In this context we do not interpret these small differences between nuclear and
515 mitochondrial phylogenies as evidence of mitochondrial introgression. Mitochondrial introgression
516 would be a very specific marker of hybridisation between populations and has been described in
517 trypanosomatids, including *T. cruzi* (Messenger et al. 2012) and in many other organisms
518 (Harrison and Larson 2014). Different ancestries between mitochondrial and nuclear genomes
519 would not be expected between LdDM62 and the recrudescent infection LdDM299 in that they
520 are likely to be the product of a single hybridisation event, based on near identical genomic
521 structure and SNP profiles.
522
523 Current understanding regarding pattern and process of hybridisation in *Leishmania* is
524 incomplete. Analysis of populations to detect and describe genomic variation in evolutionary
525 recent hybrid isolates can confirm that hybridisation occurs in natural populations and provide
526 insight into rates and patterns of recombination. For example, previous estimates based on
527 genomic analysis form natural *L. infantum* isolates from Turkey indicate a hybridisation frequency
528 of 1.3 x10^{-5} meioses per mitosis (Rogers et al. 2014). However characterisation of natural systems
529 presents particular challenges: while co-localised isolates similar to the putative parents can
530 sometimes be found, this is not guaranteed (Rogers et al. 2014). The number of independent
531 meioses sampled in a natural population can be small and is consistently difficult to quantify. The
532 recent ability to derive experimental hybrids in *L. major* (Akopyants et al. 2009) and now *L.*
533 *donovani* (Sadlova et al. 2011); Yeo et al. unpublished data) can facilitate our understanding, as
534 multiple replicated offspring from identical (and known) parents, frequency and distribution of

535 cross-overs are easier to assess. Particular questions of interest might be to determine if
536 recombination tends to occur at particular localised hot-spots? If so, are they associated with
537 particular genomic features such as GC content or between polycistronic transcription units? Are
538 crossing-over events associated with particularly high SNP mutation rates (Arbeithuber et al.
539 2015). In the current data we do not observe particular clusters of SNPs absent in the parental
540 populations that could suggest this, as these data have limited power to detect these effects,
541 which would require large number of observations of independent crossing-overs between the
542 same parental haplotypes. Similarly, the contribution of gene conversion, often associated with
543 meiosis, on either SNPs or tandem gene families are difficult to infer in these natural data. It is
544 also important to note that 'parental' isolates represent here are only proxies for the true parents
545 but results are strongly suggestive of multiple recombination events in Ethiopian *L. donovani* in
546 recent evolutionary history. Encouragingly, experiments and subsequent derivation of
547 experimental hybrids from phylogenetically similar parental genotypes also suggest frequent
548 recombination in different sand fly vector species (Yeo et al, unpublished data). Experimental
549 work will produce quantitative insights to support deeper understanding of the mechanisms and
550 implications of recombination in *L. donovani* populations.
551
552 In conclusion we have presented genome-wide sequence data for putatively hybrid isolates of *L.*
553 *donovani* from human VL cases in Ethiopia, together with isolates possessing putative parental
554 like genotypes. We confirmed that 4 of the 5 putative hybrids are, indeed hybrid offspring derived
555 from strains related to these parents, but the evolutionary history of these isolates is complex:
556 representing at least 3 different histories. The haplotypic reconstructions, distribution of parent
557 distinguishing SNPs and patterns of allele sharing are consistent with the occurence of more than
558 one hybridisation event and/or intercrossing and backcrossing to parentals, which has not been
559 observed in experimental crossing experiments to date. These data thus confirm the ability of
560 *Leishmania* to hybridise extensively in natural populations. The population of *L. donovani* in

561 Ethiopia has undergone multiple rounds of hybridisation, and we predict complex patterns of
562 crossing would be revealed by a more substantial sample size. Together with progress in deriving
563 experimental hybrids there is now promise of elucidating the mechanisms and other phylo-
564 epidemiological aspects of recombination that have widespread implications regarding the
565 spread, diagnosis and control of *L. donovani* populations.
566
567 **Materials and methods**
568
569 We generated short-read paired-end sequence data for 11 isolates of *Leishmania donovani* from
570 Ethiopia (see table 1). Full details of the origin and isolation of the strains used are described
571 elsewhere (Gelanew et al. 2010, 2014). Briefly, all were visceral leishmania isolated between
572 2007 and 2009 from humans in northern Ethiopia. Of note, isolate DM299 was a relapse of DM62
573 isolated form a HIV infected patient post treatment (Libo Kemkem-Abdurafi).
574
575 DNA library preparation was performed by shearing genomic DNA into 400–600 base pair
576 fragments by focused ultrasonication (Covaris Adaptive Focused Acoustics technology; AFA Inc.,
577 Woburn, USA), standard multiplex Illumina libraries were prepared using the NEBNext DNA
578 Library Kit. The libraries were amplified with 8 cycles of PCR using Kapa HiFi DNA polymerase'
579 and were then pooled. 100bp paired-end reads were generated on the Illumina HiSeq 2000 v3
580 according to the manufacturer's standard sequencing protocol. All sequencing data for these
581 isolates are available from the ENA under project ERP106107. The LV9 strain
582 (MHOM/ET/67/HU3 also known as MHOM/ET/67/L82) was originally isolated from a VL case in
583 the Humera district in the far North of Ethiopia in 1967 (Bradley and Kirkley 1977). The JPCM5
584 strain (MCAN/ES/98/LLM877) is an *L. infantum* from Spain, isolated from a dog in 1998; BPK282
585 (MHOM/NP/03/BPK282/0cl2) was isolated from a human VL case in Nepal in 2003. Illumina
586 whole-genome data for these isolates were obtained from the ENA database, with parasite

material, sequencing approach and analysis of these data detailed in (Rogers et al. 2011) for JPCM5 and LV9, (Downing et al. 2011) for BPK282.

Reads for each isolate were mapped to the *L. donovani* LV9 reference assembly using SMALT v0.7.0.1 (Ponstigl 2010), indexing every second 13-mer (DePristo et al. 2011) and mapping repetitively with a minimum identity of 80% and maximum insert size of 1200bp, and mapping each read in the pair independently (*-x* flag). Variants were called using the HaplotypeCaller algorithm of Genome Analysis Toolkit v3.4 (DePristo et al. 2011), following best-practice guidelines (Van der Auwera et al. 2013) except as detailed below. Variant calls were first filtered to remove any overlapping with a mask generated with the GEM mappability tool (Marco-Sola et al. 2012) to identify non-unique 100bp sequences and to remove 100bp either side of any gaps within scaffolds. Subsequent filtering with the Genome Analysis Toolkit removed sites using the filtering parameters: DP >= 5*ploidy, DP <= 1.75*(chromosome median read depth), FS <= 13.0 or missing, SOR <= 3.0 or missing, ReadPosRankSum <= 3.1 AND ReadPosRankSum >= -3.1, BaseQRankSum <= 3.1 AND BaseQRankSum >= -3.1, MQRankSum <= 3.1 AND MQRankSum >= -3.1, ClippingRankSum <= 3.1 AND ClippingRankSum >= -3.1. Calls were made both assuming diploid genotypes for every chromosome across isolates, and using a somy estimated for each chromosome independently for each isolates. Somy was estimated using the EM approach described previously (Iantorno et al. 2017), and values checked by manual inspection of read depth and allele frequency data.

The whole-genome phylogeny and principal components analysis presented here were generated by using VCFtools v0.1.15 (Danecek et al. 2011) to convert the variants from GATK vcf format to the input format for plink, and then plink v1.90b3v (Purcell et al. 2007) was used for the principal components analysis and to generate pairwise distances (1 - identity by similarity). The pairwise distances were used to calculate a neighbour-joining phylogeny using the neighbor program from

phylip v3.6.9 (Felsenstein 2005). Phasing was based on identifying illumina reads and read pairs linking heterozygous sites within each isolate, using the phase command in samtools v.0.1.19-44428cd (Li et al. 2009), with a block size (k) of 15; the phasing results did not differ for other values of k tested (11, 13, or 20) except k=30, where few variants were phased and no blocks > 1kb were shared by all isolates. Note that this phasing approach identifies heterozygous sites *de novo* from read mapping data rather than using the variant calls, and reconstructs at most two haplotypes at any locus. Phylogenies for the inferred haplotypes were generated using raxmlHPC v8.2.8 (Stamatakis 2014) under a GTR+I+G model of nucleotide substitution and otherwise default parameters.

Parent-distinguishing sites were identified as those for which both parent A isolates shared an identical homozygous genotype and all four parent B isolates were homozygous for a different allele. These sites could be unambiguously assigned as being derived from one or other parent in the putative hybrid isolates, assuming these other isolates were hybrids of these parents. To extend this analysis to other sites across the genome, a Hidden Markov model (HMM) was used to classify every 100bp window along the genome of the 5 suspected hybrid isolates by likely ancestry. Three hidden ancestry states (homozygous parent A, homozygous parent B and heterozygous from each parent) were used to explain the pattern across the genome of 4 observed parent-distinguishing SNP “symbols” (homozygous A, homozygous B, heterozygous and a non-determinate symbol for windows with either no parent-distinguishing SNPs or more than one state). The 100bp window size was chosen to make the HMM computationally tractable and so that almost every window (198,679 out of 202,940 across 5 isolates) was unambiguous for the observed symbol. All transitions between hidden states were allowed, but each hidden state could emit only the corresponding observation or the non-determinate symbol. Initial transition and emission probabilities and trained parameters are shown in Supplementary table 2; the trained parameters did not depend strongly on the initial parameters. The HMM was trained

639 independently on each chromosome and isolate, and then average transition and emission
640 parameters, weighted by chromosome lengths used to infer hidden states. Training and Viterbi
641 decoding of the HMM was performed using the HMM package in R v3.3.0 (R core team 2016).
642
643 kDNA maxicircle genome sequences were generated by mapping illumina sequence data against
644 the available maxicircle sequence assembly for *L. tarentolae* (Simpson et al. 1987) and using
645 MITObim (Hahn, Bachmann, and Chevreux 2013) to perform iterative guided assembly with block
646 size (k parameter) of 61 and with read trimming. This produced assemblies of between 19,611bp
647 and 21,682bp in a single contig in each isolate (the *L. tarentolae* maxicircle is 20,992bp), including
648 the entire transcribed region: tests using less strict criteria for assembly produced longer but less
649 reliable assemblies. The assembled contigs were then rotated using CSA (Fernandes, Pereira,
650 and Freitas 2009) before aligning with MAFFT v7.205 (Katoh and Standley 2013) with automated
651 algorithm choice (--automated1 flag); the alignment was then trimmed with trimAl v1.4
652 (Fernandes, Pereira, and Freitas 2009; Capella-Gutiérrez, Silla-Martínez, and Gabaldón 2009).
653 A maximum-likelihood phylogeny was inferred using raxmlHPC v8.2.8 under a GTR+I+G model
654 of nucleotide substitutions (Stamatakis 2014) with 10 random addition-sequence replicates, and
655 confidence in branches of the tree assessed with 500 bootstrap replicates.
656
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659
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663
664

665 **References**

666

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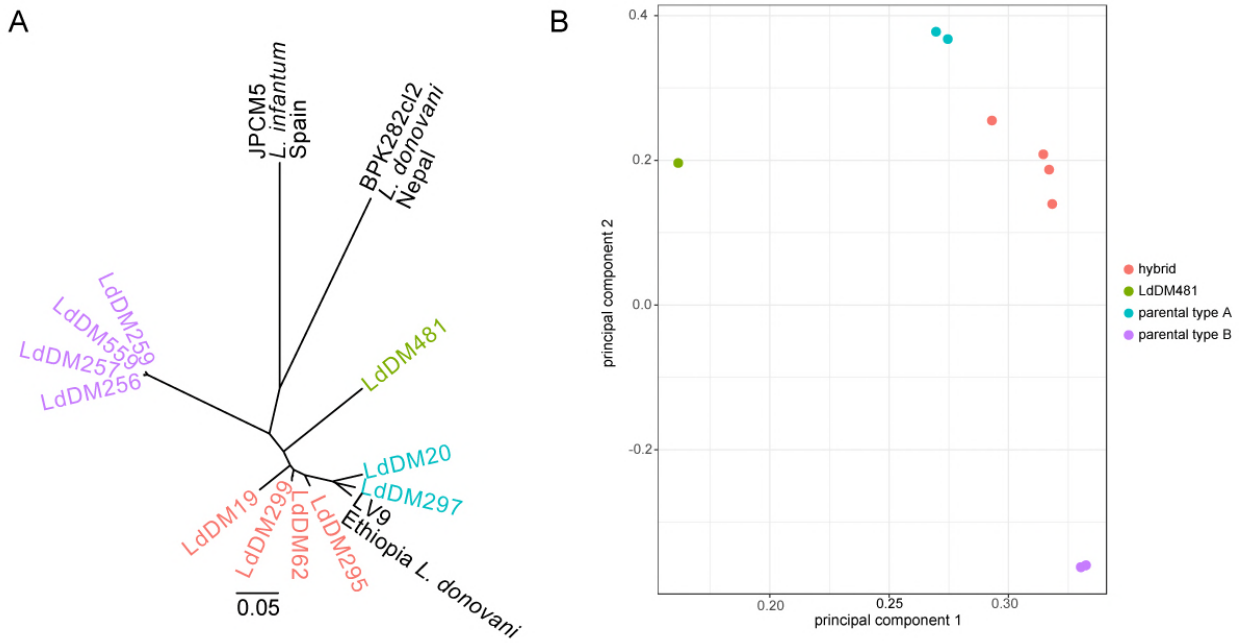
916 **Figures**

917



918

919 **Figure 1.** Neighbour-joining phylogeny (A) and principal components analysis (PCA; B) based on
920 genome-wide SNP variation data among Ethiopian *L. donovani* isolates, including additional
921 isolates from the *L. donovani* species complex. Scale bar on (A) represents genetic distances in
922 terms of expected substitutions per nucleotide site.

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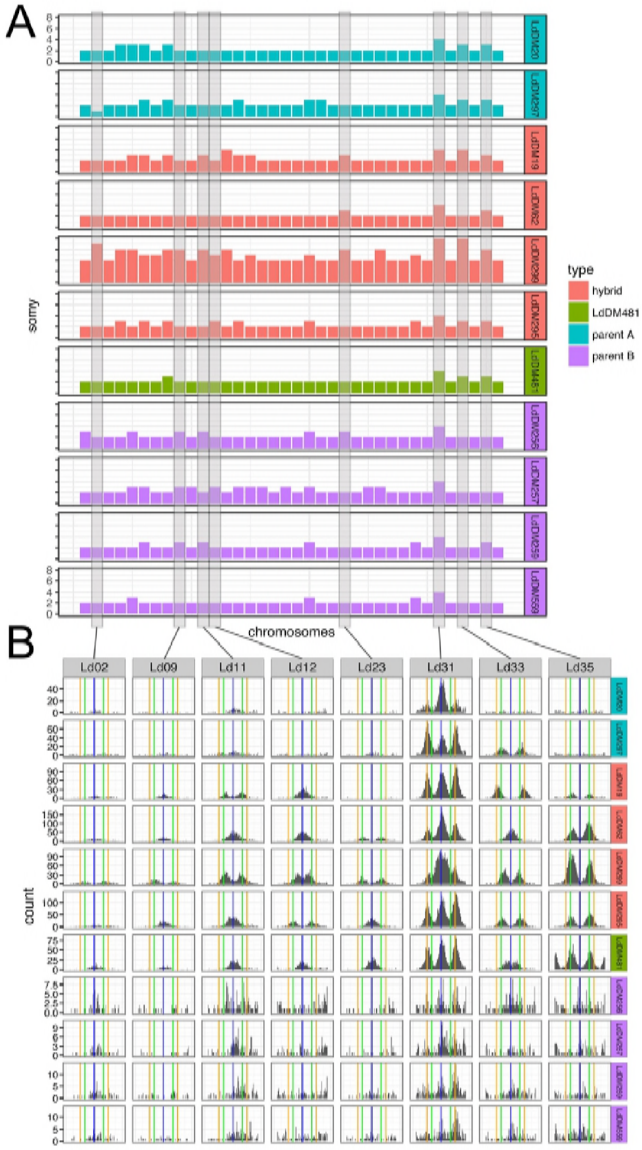


Figure 2. Variable somy across Ethiopian isolates inferred from coverage and allele frequencies. (A) Shows the inferred chromosome copy number (somy) for each chromosome across Ethiopian isolates under study. Y-axis scales are the same across all panel A rows. As detailed in methods, relative somy is inferred from the coverage depth of reads mapped to each chromosome, while the baseline somy is determined from the allele frequency distribution. (B) Shows example distributions of non-reference allele frequencies for each isolate, highlighting differences in somy. Vertical lines are at allele frequencies of 0.5 (blue), 0.33 and 0.67 (green), 0.25 and 0.75 (orange); expected for disomic, trisomic and tetrasomic chromosomes respectively.

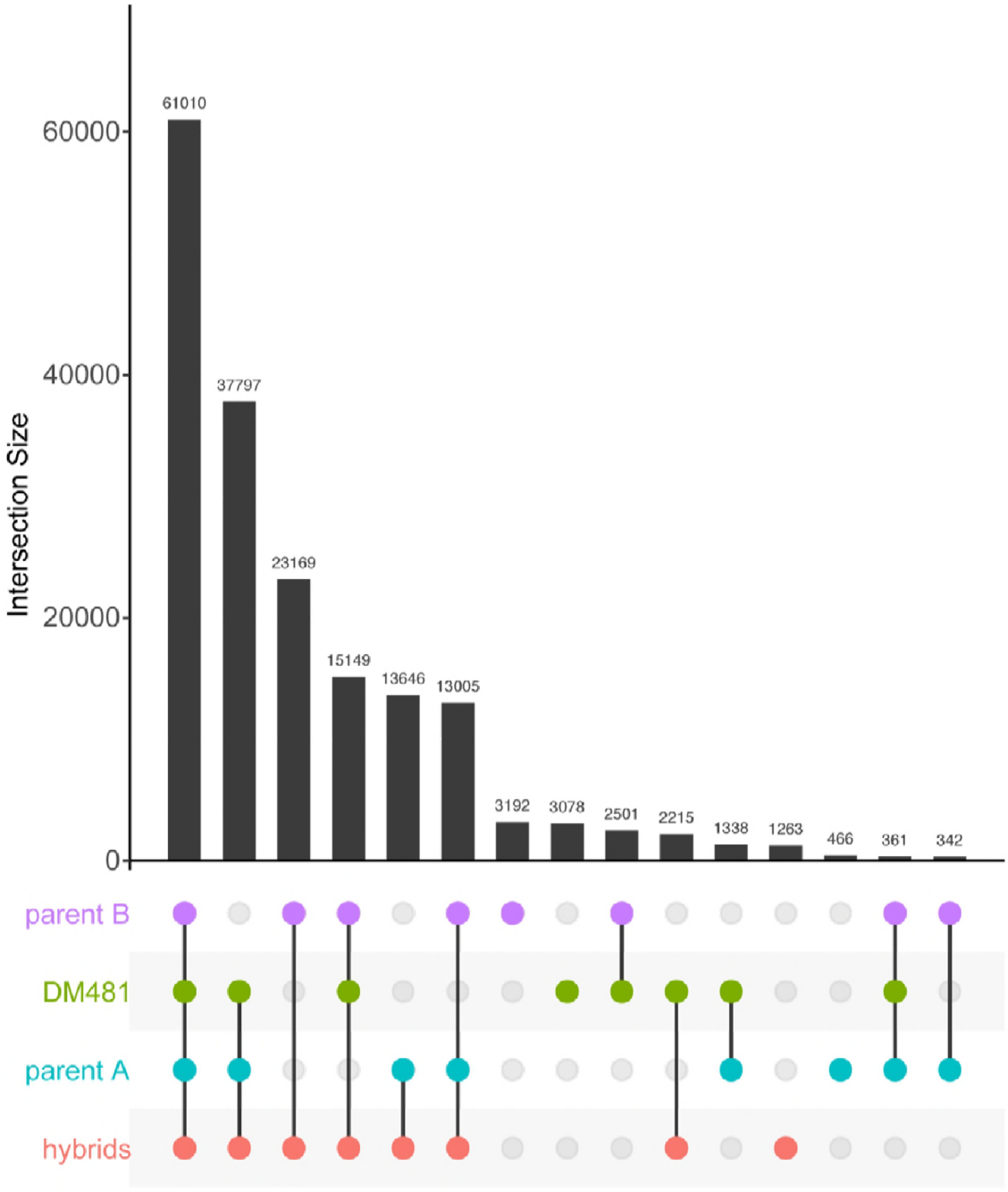
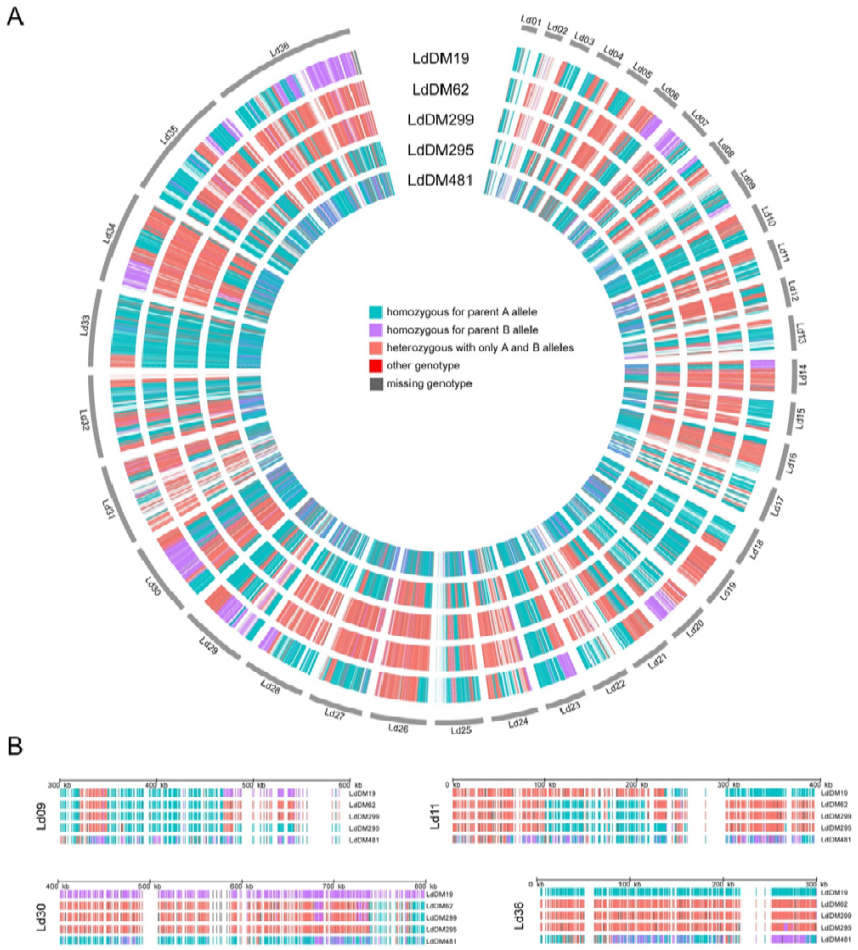


Figure 3. Pattern of allele sharing between groups of Ethiopian *L. donovani* isolates. Each row corresponds to one of the categories of isolates, with columns corresponding to non-reference alleles present in two categories or more (intersections), and the bar graph depicts the number of alleles present in at least one of the isolates in a group for each intersection.



943

944 **Figure 4.** Distribution of SNPs distinguishing between potential parents. (A) Coloured bars in
945 concentric rings represent every SNP that are fixed homozygous differences between the two
946 sets of putative parents, colored to represent the diploid genotype call as homozygous for either
947 parental type or heterozygous with one of each allele. A small number of sites had other
948 genotypes or no reliable genotype call. There are very few SNPs in the red or grey categories.
949 (B) Shows a magnified view of the same data for four regions, chosen to highlight variation
950 between different isolates.

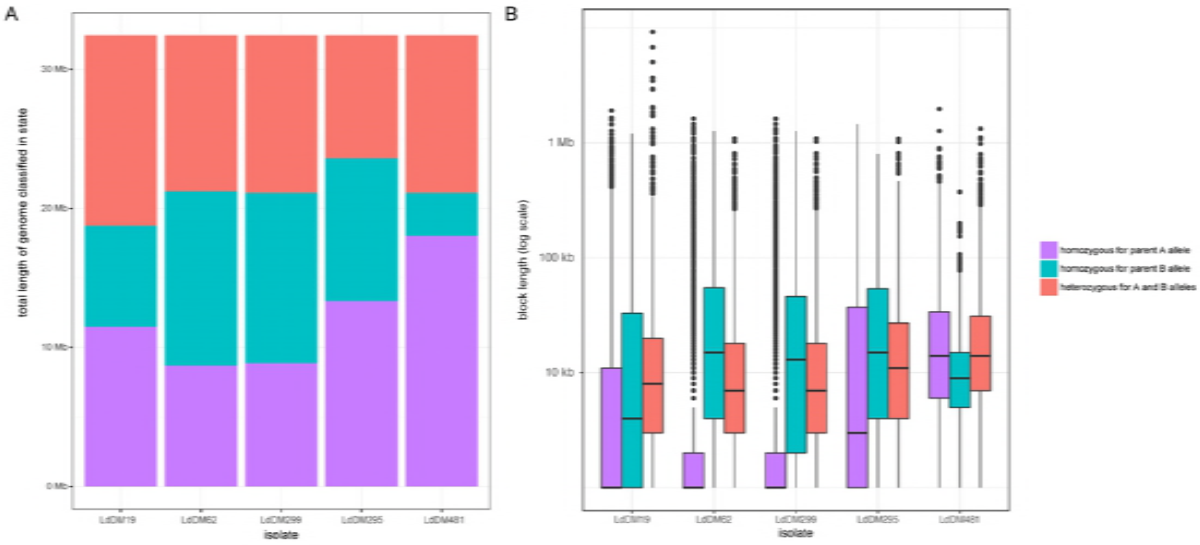


Figure 5. Distribution of genomic regions of putative hybrid isolates between parental origins based on Hidden Markov Model. (A) Shows the total number of basepairs assigned as homozygous parent A, homozygous parent B and heterozygous based on the maximum posterior probability assignment of hidden states of the Hidden Markov Model. (B) Box-and-whisker plot showing the distribution of lengths of contiguous blocks assigned to each of these three parentage states across the 5 putative hybrid strains in the most probable path identified in the Viterbi decoding. Boxes show median length and interquartile range on a log axis, whiskers are

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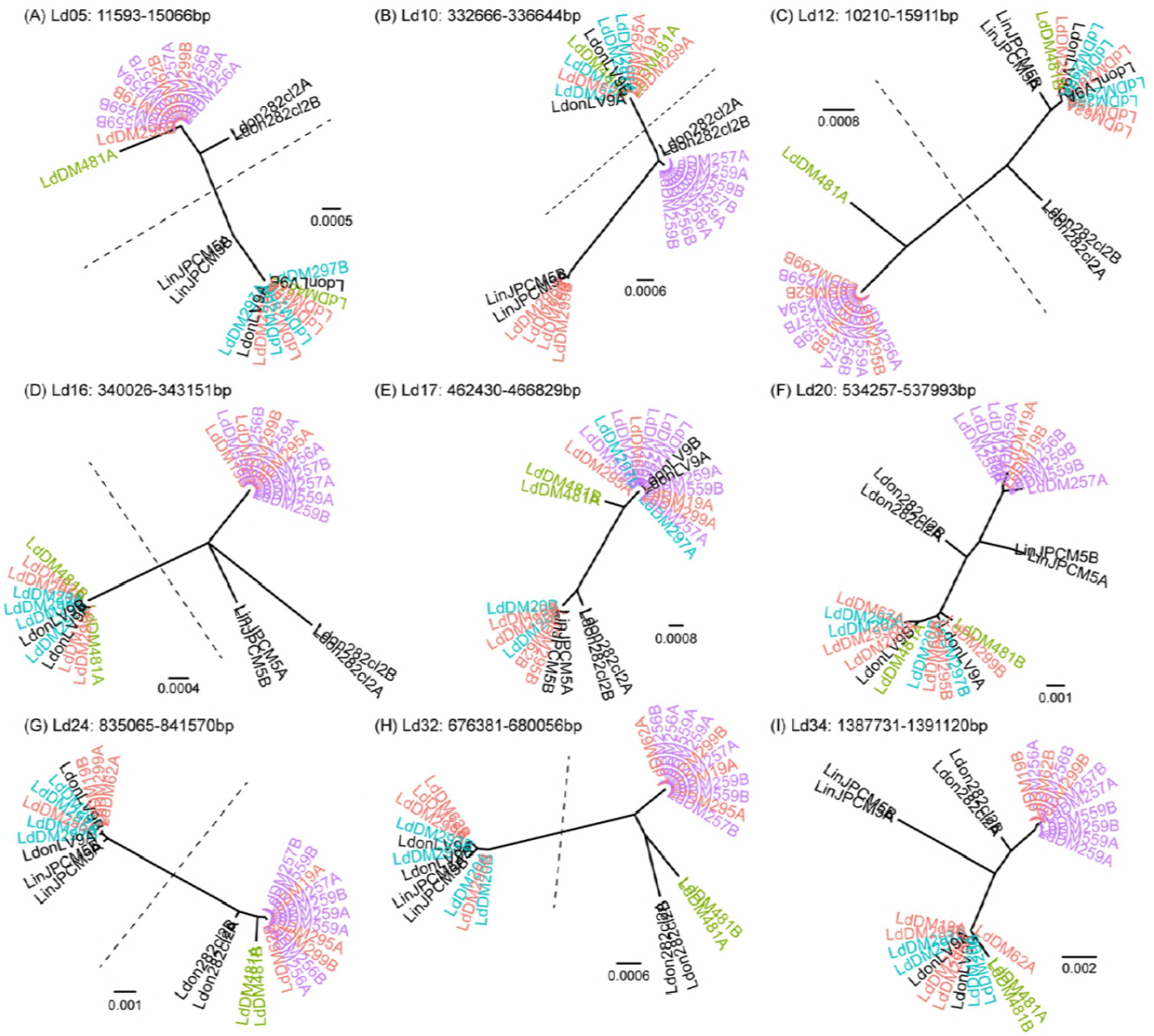
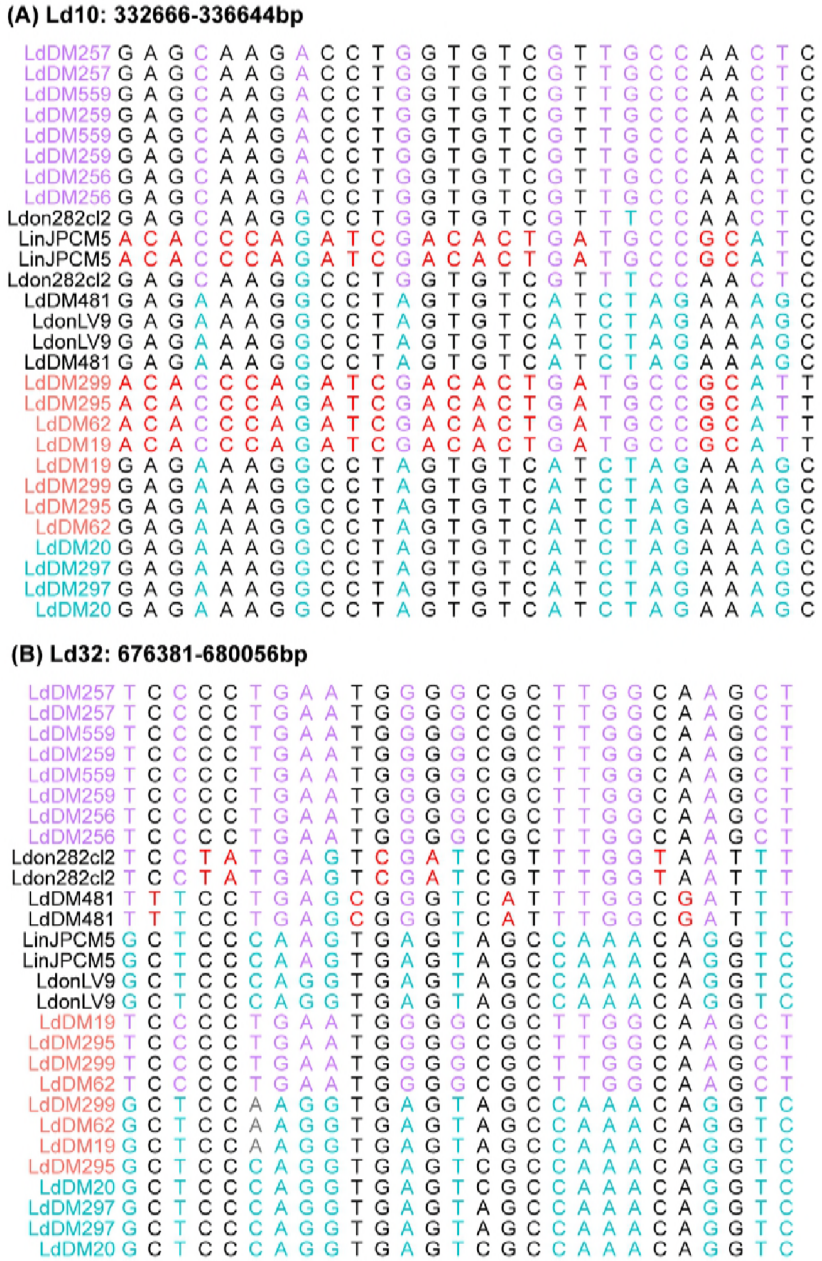


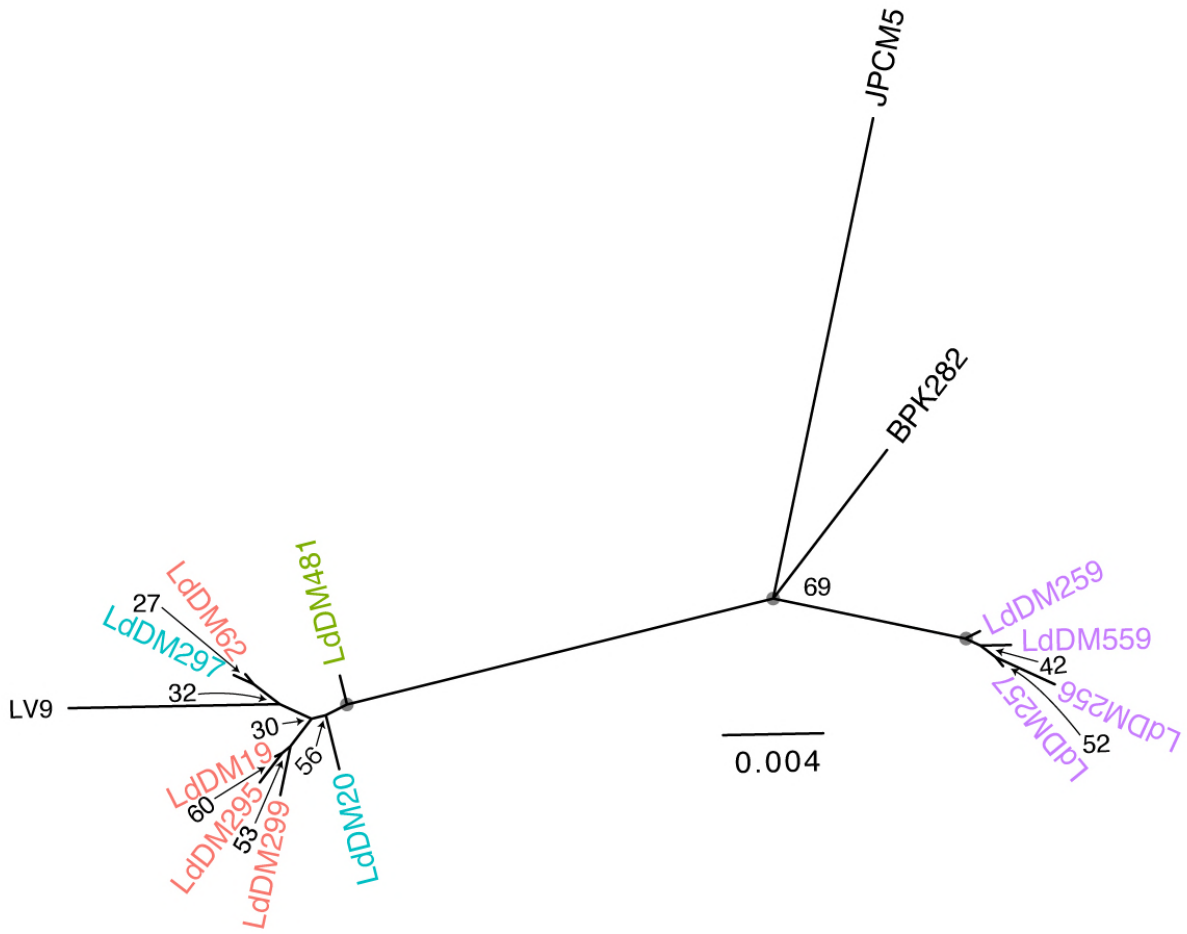
962

963 **Figure 6**. Maximum-likelihood phylogenies for inferred haplotypes at 9 genome regions at which
964 all isolates could be phased into blocks of length greater than 3kb and with an average of at least
965 4 heterozygous sites per isolate. A and B labels on the leaves are arbitrary names for the two
966 different haplotypes at each locus, for each isolate. Dotted lines separate the two hybrid
967 haplotypes for those blocks at which all the hybrid isolates (except Ld481) have one haplotype
968 from each putative parental population.



969

970 **Figure 7**. Alignments of inferred haplotypes in fully-phased regions, showing unusual haplotypes
971 in (a) the hybrid isolates and (b) LdDM481. Panels show all variable sites within two of the 'fully
972 phased' genome regions shown on figure 5. In all panels, red sites identify alleles specific to two
973 unusual haplotypes discussed in the text. Cyan and magenta identify sites at which parent A and
974 parent B isolates share fixed different alleles (parent distinguishing sites).



975

976 **Figure 8.** Maximum-likelihood phylogeny of reconstructed mitochondrial (kDNA maxicircle)
977 haplotypes. Values on nodes are bootstrap support values for the partition induced by deleting
978 the edge below each node, grey circles denote 100% support.

979

980

981 **Supplementary table 1.** Large (> 100bp) Structural variation between isolates (* this does not
982 include LV9).
983

	Number of variants called	variants segregating in recent Ethiopian isolates*	Average heterozygosity in parent A isolates	Average heterozygosity in parent B isolates	Average heterozygosity in putative hybrids	Heterozygosity of Ld481	Average heterozygosity of outgroups
Duplications	169	95	0.64	0.60	0.68	0.69	0.15
Deletions	368	279	0.61	0.49	0.62	0.60	0.23
Inversions	282	147	0.63	0.56	0.69	0.65	0.17
Insertions	1	0	0	0	0	0	0
Translocations	264	123	0.44	0.36	0.42	0.44	0.16

984

Supplementary table 2. Initial transition (a) and emission (b) probability matrix and trained transition (c) and emission (d) probabilities for HMM. NA represents “Not Allowed” emissions from that state.

(a)

From \ To	A	B	Het
A	0.8	0.1	0.1
B	0.1	0.8	0.1
Het	0.05	0.05	0.9

(b)

State \ Symbol	A	B	Het	Non-determinate
A	0.46	NA	NA	0.54
B	NA	0.46	NA	0.54
Het	NA	NA	0.46	0.54

(c)

From \ To	A	B	Het
A	0.94105911	0.03861683	0.02032406
B	0.08776222	0.88229226	0.02994551
Het	0.04503840	0.04030465	0.91465696

(d)

State \ Symbol	A	B	Het	Non-determinate
A	0.1592378	NA	NA	0.8407622
B	NA	0.1653358	NA	0.8346642
Het	NA	NA	0.0878155	0.9121845

Supplementary figure 1. Overlaid DNA histograms for selected cloned *Leishmania* strains illustrating comparable (2*n*) DNA content representative of all hybrids (grey histogram) and both parental groups (cyan and red histogram). Gates were created for G1-0 (2*n*) peaks and for G2-M (4*n*) peaks. Each strain was tested in triplicate at a minimum and a control *Leishmania* strain was included in each run as an internal standard. Relative DNA content values were calculated as a ratio compared with the internal standard. Mean G1-0 values were taken to infer relative DNA content. The x-axes represent fluorescence intensity (arbitrary units) and the y-axes represent number of events in each channel.