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Genome assemblies across the diverse evolutionary spectrum of *Leishmania* protozoan parasites

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Abstract

We report high-quality draft assemblies and gene annotation for 13 species and/or strains of the protozoan parasite genera *Leishmania, Endotrypanum* and *Crithidia*, which span the phylogenetic diversity of the Leishmaniinae sub-family within the kinetoplastid order of the phylum Euglenazoa. These resources will support studies on the origins of parasitism.

Leishmania species are widespread parasites of mammals transmitted by biting insects. Over 1.7 billion people worldwide are at risk, with hundreds of millions of people infected (1-4). The genus comprises more than 50 species, which are primarily zoonotic but in humans cause disease ranging from mild cutaneous lesions to more disseminated forms, to fatal visceralizing disease (5). While parasitism by *Leishmania* has been intensively studied, the species-specific factors that enable mammalian or insect host infections are less well understood. To provide a broad phylogenetic snapshot, we selected a spectrum of species and strains across the Leishmaniinae sub-family (5), targeting lineages within the subgenera *Leishmania*, *Viannia*, and *Mundinia* , as well as allied *Endotrypanum* and the outgroup *Crithidia fasciculata* (Table 1).

Parasites were cultivated in M199 or Schneider's medium (6) and grown to late log phase, before harvesting, lysis, and DNA purification by phenol/chloroform extraction and/or banding in CsCl gradients (to remove mitochondrial maxi- or minicircle DNA). Sequencing libraries were generated using the Illumina paired-end DNA sample preparation kit (PE-102-1001) according to the manufacturer's directions. Fragment libraries of 3 and 8kb were prepared using protocols for 454 sequencing (Roche Life Sciences). Sequencing was performed on either a 454 GS FLX Titanium (average read length of 305bp; Roche 454 Life Sciences), or Illumina GAIIx and HiSeq2000 instruments (paired end 100bp read length), except for *Crithidia* which additionally utilized long-reads generated on a RSII instrument (P5/C3 chemistry; Pacific BioSciences) (7). Total sequence genome coverage on the Illumina GAIIX or HiSeq 2000 instrument was on average 105x with tiered library insert sizes (50x fragments, 45x 3kb, and 10x 8kb and 0.05x 40kb). For all Illumina sequences we used the read processing steps within the ALLPATHS-LG (8) software prior to *de novo* assembly, which incorporates read error correction methods described by Pevzner *et al* (9). Genome assemblies were performed with default parameters using Newbler v2.0.1 (10) for 454 reads, ALLPATHS-LG (8) for Illumina reads, or HGAP version 3 (11) for long-reads (Table 1). Contigs and scaffolds were organized into pseudochromosomes using ABACAS2 (https://github.com/satta/ABACAS2), a successor to ABACAS (12), by alignment to the *Leishmania major* Friedlin genome, with the exception of *L.*

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braziliensis M2903 and *L. panamensis*, which were aligned to the *L. braziliensis* M2904 genome. The estimated haploid genome sizes ranged from 30.4 to 41.3 megabases (13).

Gene annotations were performed using the comprehensive Companion tool which incorporates a variety of *de novo* prediction criteria, as well as information from closely related genomes when available (14). The number of protein-coding genes predicted ranged from 8,285 to 9,619, typical of other *Leishmania* (13). Full annotations, as well as a variety of tools for the visualization or analysis of these genomes, are available from TriTrypDB (www.tritrypdb.org).

Public availability and accession number(s). Assemblies are deposited in the NCBI GenBank Repository under the Bioproject numbers in Table 1, including links to the primary data and annotations (PRJNA50303, PRJNA50301, PRJNA192717, PRJNA192712, PRJNA192710, PRJNA169676, PRJNA169673, PRJNA165955, PRJNA165959, PRJNA192711, PRJNA192703, PRJNA165953, PRJNA165885). Chromosome builds are available through the TriTrypDB portal (http://tritrypdb.org/tritrypdb).

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Table 1. Description of *Leishmania* species and strains including assembly parameters and links.

Subgenus	Species	strain ID	WHO Code	Source	Provenance	NCBI GenBank Bioproject[1]	Sequencing Platforms	Assembler	Contigs	N50 contig length	Assembly size (bp)	%GC	Pseudo-chromo-somes	Protein coding genes	Other assemblies
L. Leishmania	*L. major*	SD75.1 (clone)	MHOM/SN/74/SD	human, cutaneous	D. Sacks, Bethesda USA	PRJNA50303	454, Illumina	Newbler	891	95,380	31,727,271	59.3	36	8,818	
		LV39 (clone5)	MHOM/Sv/59/P	human, cutaneous	R. Titus, Boston, USA	PRJNA50301	454, Illumina	Newbler	1,667	71,452	31,961,985	59.5	36	8,971	
	L. gerbilli	LEM452	MRHO/CN/60/GERBILLI	gerbil	P. Bastien, J-P Dedet, Montpellier, France	PRJNA192717	454, Illumina	Allpaths	1,248	57,008	30,822,621	59.6	36	8,599	
	L. turanica	LEM563	MMEL/SU/79/MEL	gerbil	P. Bastien, J-P Dedet, Montpellier, France	PRJNA192712	454, Illumina	Allpaths	1,669	39,210	30,876,294	59.5	36	8,608	
	L. arabica	LEM1108	MPSA/SA/83/JISH220	*Psammomys*	P. Bastien, J-P Dedet, Montpellier, France	PRJNA192710	454, Illumina	Allpaths	1,530	52,119	30,774,332	59.2	36	8,646	
	L. tropica	L590	MHOM/IL/1990/P283	human, cutaneous	C. Jaffe, Jerusalem, Israel	PRJNA169676	454, Illumina	Allpaths	1,938	32,739	31,326,083	59.6	36	8,824	(12)
	L. aethiopica	L147	MHOM/ET/1972/L100	human, diffuse cutaneous, relapsing	C. Jaffe, Jerusalem, Israel	PRJNA169673	454, Illumina	Allpaths	1,758	38,498	31,026,739	60.1	36	8,722	
L. Viannia	*L.braziliensis*	M2903	MHOM/BR/72/M2903	human, cutaneous	J. Shaw, Brasil	PRJNA165955	454, Illumina	Newbler	3,934	61,918	32,590,753	57.4	35	9,269	
	L. panamensis	L13	MHOM/COL/81/L13	human, mucosal	N. Saravia, Cali, Colombia	PRJNA165959	454, Illumina	Allpaths	3,163	22,576	31,108,242	57.4	35	8,665	
L. Mundinia	*L. enriettii*	LEM3045	MCAV/BR/95/CUR3	*Cavia porcellus* guinea pig	P. Bastien, J-P Dedet, Montpellier, France	PRJNA192711	454, Illumina	Allpaths	1,171	102,666	30,427,298	59.3	36	8,731	(14)
	L. martiniquensis	LEM2494	MHOM/MQ/92/MAR1	human, diffuse cutaneous, HIV	P. Bastien, J-P Dedet, Montpellier, France	PRJNA192703	454, Illumina	Allpaths	628	147,290	30,528,357	59.6	36	8,483	(14)
Endotrypanum	*Endotrypanum monterogei*	LV88	none	*Choloepus hoffmani* sloth	Michael Chance, Liverpool UK	PRJNA165953	454, Illumina	Newbler	3,517	33,059	32,086,870	52.5	36	8,285	(13)
Crithidia	*Crithidia fasciculata*	Cf-C1 (clone)	none	mosquito	Larry Simpson, Los Angeles, USA	PRJNA165885	454, Illumina, PacBio	HGAP	494	778,443	41,297,378	57.0	30	9,619	(8)

Footnote 1 Each bioproject link contains links to the current assembly, primary data sets, and other relevant information.