AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequences of *Leishmania* (*Leishmania*) *amazonensis*, *Leishmania* (*Leishmania*) *mexicana*, and *Leishmania* (*Leishmania*) *aethiopica*, Potential Etiological Agents of Diffuse Cutaneous Leishmaniasis

Dhwani Batra,[b] Wuling Lin,[a,c] Vidhya Narayanan,[a,c] Lori A. Rowe,[b] Mili Sheth,[b] Yueli Zheng,[a,d] Vladimir Loparev,[b] Marcos de Almeida[a]

[a]Reference Diagnostic Laboratory, Parasitic Diseases Branch/Department of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[b]Biotechnology Core Facility Branch, DSR/NCEZID, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[c]IHRC, Inc., Atlanta, Georgia, USA

[d]Eagle Global Scientific, San Antonio, Texas, USA

ABSTRACT We present here the draft genome sequences of *Leishmania* (*Leishmania*) *amazonensis*, *Leishmania* (*Leishmania*) *mexicana*, and *Leishmania* (*Leishmania*) *aethiopica*, potential etiological agents of diffuse cutaneous leishmaniasis (DCL). Sequence data were obtained using PacBio and MiSeq platforms. The PacBio assemblies generated using Canu v1.6 are more contiguous than are those in the available data.

Leishmaniasis is a vector-borne disease caused by more than 20 species of parasites in the *Viannia* and *Leishmania* subgenera, affecting millions of people yearly (1, 2). Cutaneous leishmaniasis (CL) is the most prevalent clinical manifestation, which usually heals spontaneously within 6 months to 3 years. Diffuse CL (DCL) is a rare and poorly understood form of CL, characterized by non-self-healing chronic lesions in large areas of the skin, with high parasite proliferation and resistance to most therapeutic agents (3–5). DCL is caused mostly by strains of *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania* (*Leishmania*) *mexicana* in Latin America and *Leishmania* (*Leishmania*) *aethiopica* in the Horn of Africa. Treatment of the primary CL is required to prevent the morbidity and mortality of leishmaniasis, but the choice and effectiveness of the therapy depend on the parasite species and the host immune response (1, 5–7). Therefore, species-specific diagnostic methods are crucial for clinical management.

To increase the quality of *Leishmania* species reference sequences, we submitted to the database the draft genome sequences of the species *L.* (*L.*) *amazonensis*, *L.* (*L.*) *mexicana*, and *L.* (*L.*) *aethiopica*. The parasites listed in Table 1 were identified as infecting species of three CL clinical cases from French Guyana, Texas, and Ethiopia, respectively, using the CDC's current diagnostic approach (8, 9). A portion of each CL specimen was cultivated at 26°C in 20 ml RPMI medium (Invitrogen, GA) enriched with 0.5% HEPES, 0.25% L-glutamine, 0.02% folic acid, 15% fetal bovine serum, 0.02% hemin, 1% minimal essential medium (MEM) vitamin solution (100×), penicillin-streptomycin (75 I/ml to 75 μg/ml), and gentamicin (50 μg/ml) (pH 7.4). When the parasites reached log phase, 1.5-ml aliquots of the culture were preserved with 10% dimethyl sulfoxide (DMSO) in liquid nitrogen. For this study, parasites were reactivated by mixing the thawed culture aliquots with 20 ml of fresh RPMI medium. After 2 to 3 weeks of incubation, 1.0 ml of culture aliquots was centrifuged at 6,000 × g for 15 min, and the pellet was used for DNA extraction using the MagAttract high-molecular-weight (HMW) DNA kit (Qiagen, MD).

Citation Batra D, Lin W, Narayanan V, Rowe LA, Sheth M, Zheng Y, Loparev V, de Almeida M. 2019. Draft genome sequences of *Leishmania* (*Leishmania*) *amazonensis*, *Leishmania* (*Leishmania*) *mexicana*, and *Leishmania* (*Leishmania*) *aethiopica*, potential etiological agents of diffuse cutaneous leishmaniasis. Microbiol Resour Announc 8:e00269-19. https://doi.org/10.1128/MRA.00269-19.

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

Address correspondence to Marcos de Almeida, MdeAlmeida@cdc.gov.

Received 8 March 2019
Accepted 25 April 2019
Published 16 May 2019

TABLE 1 Assembly metrics for *L.* (*L.*) *amazonensis*, *L.* (*L.*) *mexicana*, and *L.* (*L.*) *aethiopica* and their corresponding reference strains

Strain	Read type(s)	Total length (bp)	No. of scaffolds	No. of contigs	N_{50} length (bp)	L_{50}	Longest contig (bp)	No. of Illumina reads	No. of PacBio reads[a]	G+C content (%)	Coverage (×)
L. (*L.*) *amazonensis* CDC210-L1346	PacBio, Illumina	33,504,997	92	92	850,106	12	3,425,950	30,427,462	262,667	59.71	75
L. (*L.*) *amazonensis* M2269[b]	454, Illumina	29,029,348	2,627	2,944	19,306	430	113,027	NA	NA	59.26	NA
L. (*L.*) *mexicana* CDC215-L49	PacBio, Illumina	32,057,209	55	55	825,953	11	3,409,057	30,816,844	323,584	59.79	87
L. (*L.*) *mexicana* U1103[b]	Sanger, Illumina	32,108,741	588	927	164,930	56	1,024,353	NA	NA	59.8	NA
L. (*L.*) *aethiopica* CDC209-L1204	PacBio, Illumina	33,648,436	118	118	763,733	15	1,670,940	10,767,274	245,491	60.38	74
L. (*L.*) *aethiopica* L147[b]	Illumina	31,630,816	160	1,698	39,581	240	168,996	NA	NA	60.07	NA

[a] NA, not available.

[b] Indicates currently available assemblies in the public databases.

The DNA extracts were used for the preparation of Illumina MiSeq and PacBio libraries. Dual-indexed libraries using NEBNext Ultra library prep (New England BioLabs, MA) and barcoding indices were manufactured at the CDC-Biotechnology Core Facility. These libraries were sequenced on a MiSeq platform (Illumina, CA) using the MiSeq 2 × 250-cycle sequencing kit. Sequence reads were filtered, base called, and demultiplexed using bcl2fastq (v2.19), with default parameters. PacBio libraries were prepared using the standard PacBio 20-kb procedure (Pacific Biosciences, CA). The libraries were size selected using BluePippin (Sage Science, MA), bound to the polymerase using the DNA/polymerase binding kit P6 v2, loaded onto 3 to 5 single-molecule real-time (SMRT) cells, and sequenced with C4 v2 chemistry for 360-min movies on the RS II instrument (Pacific Biosciences).

The filtered PacBio (minlength = 1,000) reads were *de novo* assembled using Canu v1.6 (genomeSize = 32m) (10). After removing contigs with a coverage of <10×, the remaining contigs were corrected with Illumina reads using unicycler_polish (default parameters; Unicycler package v4.4) (11). As shown in Table 1, the resultant assemblies are more contiguous than are those in the data available in GenBank for *L.* (*L.*) *amazonensis* LeiAma1.0 (GCA_000438535) (12), *L.* (*L.*) *mexicana* ASM23466v4 (GCA_000234665) (13), and *L.* (*L.*) *aethiopica* L147 (GCA_000444285).

Data availability. The genome contigs have been deposited in GenBank under accession numbers RZOD00000000 [*L.* (*L.*) *amazonensis* CDC210-L1346], RZOC00000000 [*L.* (*L.*) *mexicana* CDC215-L49], and RZOE00000000 [*L.* (*L.*) *aethiopica* CDC209-L1204] under BioProject number PRJNA484340. The accession numbers for the raw reads are SRR8377732, SRR7867275, SRR7867276, SRR7867277, SRR8377734, SRR7867272, SRR7867273, SRR7867285, SRR7867284, SRR8377733, SRR7867278, SRR7867279, SRR7867280, SRR7867281, and SRR7867274.

ACKNOWLEDGMENTS

This work was made possible through support from the CDC's Advanced Molecular Detection (AMD) program.

We acknowledge Stephen M. Beverley and The Genome Institute, Washington University School of Medicine, for publication of the *L.* (*L.*) *aethiopica* L147 genome sequence used as a reference in this publication.

REFERENCES

1. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, Carvalho E, Ephros M, Jeronimo S, Magill A. 2017. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Am J Trop Med Hyg 96:24–45. https://doi.org/10.4269/ajtmh.16-84256.
2. Herwaldt BL. 1999. Leishmaniasis. Lancet 354:1191–1199. https://doi.org/10.1016/S0140-6736(98)10178-2.
3. França-Costa J, Wanderley JL, Deolindo P, Zarattini JB, Costa J, Soong L, Barcinski MA, Barral A, Borges VM. 2012. Exposure of phosphatidylserine on Leishmania amazonensis isolates is associated with diffuse cutaneous leishmaniasis and parasite infectivity. PLoS One 7:e36595. https://doi.org/10.1371/journal.pone.0036595.
4. Hashiguchi Y, Gomez EL, Kato H, Martini LR, Velez LN, Uezato H. 2016. Diffuse and disseminated cutaneous leishmaniasis: clinical cases experienced in Ecuador and a brief review. Trop Med Health 44:2. https://doi.org/10.1186/s41182-016-0002-0.
5. van Griensven J, Gadisa E, Aseffa A, Hailu A, Beshah AM, Diro E. 2016. Treatment of cutaneous leishmaniasis caused by Leishmania aethiopica: a systematic review. PLoS Negl Trop Dis 10:e0004495. https://doi.org/10.1371/journal.pntd.0004495.
6. Cañeda-Guzmán IC, Salaiza-Suazo N, Fernández-Figueroa EA, Carrada-Figueroa G, Aguirre-García M, Becker I. 2014. NK cell activity differs between patients with localized and diffuse cutaneous leishmaniasis infected with Leishmania mexicana: a comparative study of TLRs and cytokines. PLoS One 9:e112410. https://doi.org/10.1371/journal.pone.0112410.
7. Coelho AC, Trinconi CT, Costa CH, Uliana SR. 2016. In vitro and in vivo

miltefosine susceptibility of a Leishmania amazonensis isolate from a patient with diffuse cutaneous leishmaniasis: follow-up. PLoS Negl Trop Dis 10:e0004720. https://doi.org/10.1371/journal.pntd.0004720.
8. de Almeida ME, Koru O, Steurer F, Herwaldt BL, da Silva AJ. 2017. Detection and differentiation of Leishmania spp. in clinical specimens by use of a SYBR green-based real-time PCR assay. J Clin Microbiol 55:281–290. https://doi.org/10.1128/JCM.01764-16.
9. de Almeida ME, Steurer FJ, Koru O, Herwaldt BL, Pieniazek NJ, da Silva AJ. 2011. Identification of Leishmania spp. by molecular amplification and DNA sequencing analysis of a fragment of rRNA internal transcribed spacer 2. J Clin Microbiol 49:3143–3149. https://doi.org/10.1128/JCM.01177-11.
10. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:722–736. https://doi.org/10.1101/gr.215087.116.
11. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
12. Real F, Vidal RO, Carazzolle MF, Mondego JM, Costa GG, Herai RH, Wurtele M, de Carvalho LM, Carmona e Ferreira R, Mortara RA, Barbieri CL, Mieczkowski P, da Silveira JF, Briones MR, Pereira GA, Bahia D. 2013. The genome sequence of Leishmania (Leishmania) amazonensis: functional annotation and extended analysis of gene models. DNA Res 20:567–581. https://doi.org/10.1093/dnares/dst031.
13. Rogers MB, Hilley JD, Dickens NJ, Wilkes J, Bates PA, Depledge DP, Harris D, Her Y, Herzyk P, Imamura H, Otto TD, Sanders M, Seeger K, Dujardin JC, Berriman M, Smith DF, Hertz-Fowler C, Mottram JC. 2011. Chromosome and gene copy number variation allow major structural change between species and strains of Leishmania. Genome Res 21:2129–2142. https://doi.org/10.1101/gr.122945.111.