





## Draft Genome Sequence of the Protozoan Parasite *Leishmania* braziliensis Strain BA788, Isolated from a Clinical Case in Bahia State, Brazil

Anderson Coqueiro-dos-Santos, a Gabrielle Ariadine Bento, Aldina Barral, Camila I. de Oliveira, Daniella C. Bartholomeu

<sup>a</sup>Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Laboratório de Imunologia e Genômica de Parasitos, Belo Horizonte, Minas Gerais, Brazil <sup>b</sup>Instituto Gonçalo Moniz, Fiocruz, Salvador, Bahia, Brazil

cInstituto Nacional de Ciência e Tecnologia em Doenças Tropicais, Salvador, Bahia, Brazil

Camila I. de Oliveira and Daniella C. Bartholomeu contributed equally to this work.

**ABSTRACT** The draft genome of the parasite *Leishmania braziliensis* strain BA788, which was isolated from a patient from Bahia state, Brazil, was sequenced using Illumina paired-end technology. The assembled genome is 33.5 Mb long and contains 7,603 genes. This genome will contribute to studies aimed at understanding the pathogenesis caused by this parasite strain.

eishmaniasis is a complex of diseases transmitted to humans and other mammals by the bite of sand flies from the *Phlebotomus* and *Lutzomyia* genera. These diseases are endemic in 90 countries and pose a risk to 350 million people, with an estimated 1 million cases per year (1). Leishmaniasis encompasses a broad range of clinical manifestations, from localized cutaneous disease to the visceral form with potentially fatal outcomes. Distinct clinical features depend on the *Leishmania* species involved and the host immune response. *Leishmania* (*Viannia*) braziliensis is the most important etiological agent of tegumentary leishmaniasis in the Americas, with predominantly rural and peridomiciliary transmission (2).

L. braziliensis strain MHOM/BR/01/BA788 was isolated from a 16-year-old male patient from Jequié, Bahia state, northeastern Brazil, who presented with a single ulcerated lesion on the lower limb (3). The Montenegro skin test supported the diagnosis as localized cutaneous leishmaniasis. Lymph nodes close to the lesion site were aspirated, and samples were cultured in Schneider medium (Sigma Chemical Co.) supplemented with 100 U/mL of penicillin, 100  $\mu$ g/mL of streptomycin, 10% heat-inactivated fetal calf serum (Life Technologies), and 2% sterile human urine. The research was carried out in accordance with the Declaration of Helsinki. The patient underwent conventional treatment with antimonials, and the lesion was completely reepithelized after a single round of treatment. MHOM/BR/01/BA788 was registered at SisGen (https://sisgen.gov.br/paginas/pubpesqatividade.aspx) under the accession number A7A6463.

Promastigote parasites were maintained in Schneider medium (Sigma Chemical Co.) supplemented with 100 U/mL of penicillin, 100  $\mu$ g/mL of streptomycin, and 10% heat-inactivated fetal calf serum (Life Technologies). Promastigotes were grown in airtight flasks (T25 [nonvented]), incubated upright in a 25°C incubator, and passaged every 3 days (4). Genomic DNA was extracted using the Wizard genomic DNA purification kit (Promega) following the manufacturer's instructions. The DNA sample was then checked for Mycoplasma contamination by PCR amplification (5) and genotyped by PCR using L. braziliensis-specific primers (6), and its quality and integrity were assessed with ethidium bromide-stained agarose gels. A sequencing library was constructed using the Illumina TruSeq library preparation method. Paired-end sequencing (151 bp from

**Editor** Catherine Putonti, Loyola University Chicago

Copyright © 2022 Coqueiro-dos-Santos et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Daniella C. Bartholomeu, daniellaufmg@gmail.com.

The authors declare no conflict of interest.

**Received** 3 August 2022 **Accepted** 15 October 2022 **Published** 1 November 2022 each end) was performed on an Illumina HiSeq 2500 instrument at Macrogen, Inc. (South Korea).

A total of 28,378,882 reads were generated, corresponding to  $100 \times g$ enome coverage. Read quality was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/ fastgc), and low-quality reads and adapter sequences were removed with Trimmomatic v0.39 (7), based on minimal mean Phred quality scores of 30 and read lengths of >50 bp. The reads were mapped against the reference genome of L. braziliensis strain M2904 (GenBank accession number GCA 900537975.1) using BWA-MEM v0.7.17 (8); this resulted in 97.3% mapped reads, which were used for reference-guided assembly with RGAAT v2.0 (9) with the following parameters: -q 20 -l 50 -d 8 -c 5 -fm no. The assembled genome consisted of 33.5 Mb spread over 51 scaffolds, with an  $N_{50}$  value of 1,142,394 bp and a GC content of 58.05%. The annotation was transferred from L. braziliensis strain M2904 (GenBank accession number GCA\_900537975.1) by RGAAT using BLAT v35 genome comparisons (9). The genome contained 7,488 predicted protein-coding genes and 115 structural RNAs. Analyses of the predicted protein sequences with BUSCO v5.2.2 (10) showed that the proteome contained 98.5% of the 130 markers of single-copy ortholog genes that are expected to be present in 31 protozoan species. The annotation was converted to an SQN file by using the NCBI-provided script tbl2asn and was submitted to GenBank.

**Data availability.** This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number JAMFLV00000000. The Illumina raw reads have been deposited in the Sequence Read Archive (SRA) under accession number SRR15902693.

## **ACKNOWLEDGMENTS**

This work was supported by the Brazilian Federal Agency for Support of Graduate Education (CAPES), Brazilian Council for Scientific and Technological Development (CNPq), Minas Gerais State Agency for Research and Development (FAPEMIG), National Institute for Science and Technology in Vaccines (INCTV), and Fundação Oswaldo Cruz. AB, CIO and DCB are CNPq research fellows. ACS received scholarships from CNPq and GAB from CAPES. We also would like to thank Michele Matos for her technical support.

## **REFERENCES**

- World Health Organization. 8 January 2022. Leishmaniasis. https://www .who.int/news-room/fact-sheets/detail/leishmaniasis. Accessed 30 September 2022.
- Burza S, Croft SL, Boelaert M. 2018. Leishmaniasis. Lancet 392:951–970. https://doi.org/10.1016/S0140-6736(18)31204-2.
- de Moura TR, Novais FO, Oliveira F, Clarêncio J, Noronha A, Barral A, Brodskyn C, de Oliveira Cl. 2005. Toward a novel experimental model of infection to study American cutaneous leishmaniasis caused by *Leishmania braziliensis*. Infect Immun 73:5827–5834. https://doi.org/10.1128/IAI.73.9.5827-5834.2005.
- Sharma R, Avendaño Rangel F, Reis-Cunha JL, Marques LP, Figueira CP, Borba PB, Viana SM, Beneke T, Bartholomeu DC, de Oliveira CI. 2021. Targeted deletion of centrin in *Leishmania braziliensis* using CRISPR-Cas9-based editing. Front Cell Infect Microbiol 11:790418. https://doi.org/10.3389/fcimb .2021.790418.
- Timenetsky J, Santos LM, Buzinhani M, Mettifogo E. 2006. Detection of multiple mycoplasma infection in cell cultures by PCR. Braz J Med Biol Res 39:907–914. https://doi.org/10.1590/s0100-879x2006000700009.

- Castilho TM, Shaw JJ, Floeter-Winter LM. 2003. New PCR assay using glucose-6phosphate dehydrogenase for identification of *Leishmania* species. J Clin Microbiol 41:540–546. https://doi.org/10.1128/JCM.41.2.540-546.2003.
- 7. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
- 8. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324.
- Liu W, Wu S, Lin Q, Gao S, Ding F, Zhang X, Aljohi HA, Yu J, Hu S. 2018. RGAAT: a reference-based genome assembly and annotation tool for new genomes and upgrade of known genomes. Genomics Proteomics Bioinformatics 16:373–381. https://doi.org/10.1016/j.gpb.2018.03.006.
- Manni M, Berkeley MR, Seppey M, Simão FA, Zdobnov EM. 2021. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol Biol Evol 38:4647–4654. https://doi.org/10.1093/molbev/msab199.