AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Whole-Genome Sequence of *Leishmania* (*Viannia*) *braziliensis* Presenting *Leishmania RNA Virus 1*, from Western Amazon, Brazil

Andonai Krauze,[a] Raul Maia Falcão,[b] Marlon Grégori Flores Custódio,[c] Iasmin Ferreira Pimentel,[c] Lilian Motta Cantanhêde,[c] Gabriel Eduardo Melim Ferreira,[c,d] Elisa Cupolillo,[d,e] Ricardo de Godoi Mattos Ferreira[c,d]

[a]Laboratório de Genética Humana da Universidade Federal de Rondônia–UNIR, Porto Velho, Brazil
[b]Universidade Federal de Pernambuco, Recife, Brazil
[c]Laboratório de Epidemiologia Genética, Fundação Oswaldo Cruz, Fiocruz Rondônia, Porto Velho, Brazil
[d]Instituto Nacional de Epidemiologia da Amazônia Ocidental–EpiAMO, Rondônia, Brazil
[e]Fundação Oswaldo Cruz, Instituto Oswaldo Cruz–IOC, Rio de Janeiro, Brazil

ABSTRACT *Leishmania* (*Viannia*) *braziliensis* is the main etiological agent of tegumentary leishmaniasis in the neotropics. Here, we report a draft genome sequence (31.2 Mb) of an *L. braziliensis* strain from the western Amazon region of Brazil. This genome sequence will complement those available for other *Leishmania* species and contribute to further studies focusing on this parasite and the neglected diseases associated with it.

Leishmania braziliensis (Kinetoplastida: Trypanosomatidae) belongs to the subgenus *Viannia*, which comprises species found exclusively in the neotropics; it is the main agent of tegumentary leishmaniasis (TL) in this region and causes a broad range of clinical manifestations, ranging from single to multiple lesions in the skin and nasopharyngeal mucosa, as well as persistent metastatic disease. The clinical expression of TL caused by *L. braziliensis* is multifactorial, being influenced by host and parasitic characteristics, including its endosymbiosis with *Leishmania RNA virus 1* (LRV1) (1). The hypothesis that LRV1 is an ancient virus that coevolved with *Leishmania* species (2) and is not transmitted from one *Leishmania* strain to another is well accepted. Genetic clusters observed for *L. braziliensis* strains correlate with the presence/absence of LRV1, as well as with the phylogeny of this endosymbiont (3), indicating differences in the genomes of *L. braziliensis* strains bearing LRV1.

Some studies have revealed a considerable intraspecies variability in *L. braziliensis* (4, 5), which could explain its ability to adapt to different ecological conditions. There are around 30 *Leishmania* genome assemblies available, 6 corresponding to *Leishmania* (*Viannia*) species and only 2 *for L. braziliensis*. These genomes were obtained from long-term cultures and maintained using *in vivo* and *in vitro* conditions, which were recently demonstrated to affect genomic characteristics of this organism (6).

Here, we report the genome of an *L. braziliensis* strain (IOC-L3564) isolated in 2014 from a cutaneous lesion from a patient infected in the western Amazon region of Brazil and maintained with few *in vitro* passages. This is the first reported genome sequence of an *L. braziliensis* strain presenting the endosymbiont LRV1. The strain was typed as *L. braziliensis* by the *hsp*70 PCR gene restriction fragment length polymorphism protocol and isoenzyme electrophoresis (7, 8) and was deposited in the *Leishmania* collection at the Oswaldo Cruz Institute. LRV1 was detected by reverse transcriptase PCR (8). Genomic DNA was extracted from an *in vitro* culture and purified using a PureLink DNA minikit prior to library preparation. The library was prepared with an Ion Xpress Plus

Received 9 July 2018 **Accepted** 14 August 2018 **Published** 6 September 2018

Citation Krauze A, Falcão RM, Custódio MGF, Pimentel IF, Cantanhêde LM, Ferreira GEM, Cupolillo E, Ferreira RDGM. 2018. Draft whole-genome sequence of *Leishmania* (*Viannia*) *braziliensis* presenting *Leishmania RNA virus 1*, from western Amazon, Brazil. Microbiol Resour Announc 7:e00924-18. https://doi.org/10.1128/MRA.00924-18.

Editor Jason Stajich, University of California, Riverside

Address correspondence to Andonai Krauze, andonai@unir.br.

TABLE 1 Characteristics of the draft genome sequence of *L. braziliensis* strain IOC-L3564, a parasite from western Amazon, Brazil, presenting LRV1

Parameter	Results
SRA no.	SRP144244
ENA assembly no.	GCA_003304975
GenBank accession no.	QFBG00000000
Total no. of reads	3,400,000
Read length (bp)	184
Coverage (×)	17.4
Draft genome size (bp)	38,003,648
GC content (%)	57.38
No. of contigs	8,090
N_{50} contig size (bp)	7,920
No. of scaffolds	1,029
N_{50} scaffold size (bp)	758,103
Total no. of genes annotated	8,278
No. of full-length genes	8,024
No. of partial-length genes	254
No. of genes with known function	4,023
No. of tRNAs	65
No. of snRNAs	2
No. of snoRNAs	5
No. of rRNAs	10
No. of pseudogenes	935

fragment library kit. Genomic libraries were enriched using an Ion PGM template Hi-Q View OT2 kit and sequenced using the Ion Torrent platform.

Using the TMAP tool for the Ion Torrent platform (https://github.com/iontorrent/TMAP), we determined the obtained reads to have 98.39% identity to the reference genome *L. braziliensis* strain M2904 (release TriTrypDB-27) (9). Trimming under the parameters TRAILING:5 LEADING:7 SLIDINGWINDOW:3:15 MINLEN:150 was performed using Trimmomatic (10). *De novo* assembly of the trimmed reads was conducted with SPAdes version 3.1.10 (11), which generated 10,557 scaffolds. The redundant scaffolds were removed using Redundans version 0.13a (12), which resulted in 7,363 scaffolds that were oriented and ordered on ABACAS version 1.3.1 (13) with default parameters; 34 scaffolds/chromosomes were obtained as the consensus for the haploid *Leishmania* genome (90.57% coverage), while 993 scaffolds did not match the reference genome *L. braziliensis* M2904 (release TriTrypDB-27).

QUAST software was used to check the quality of the assembly and to determine genome fraction (%) metrics (14). Annotation and gene prediction were performed on the Companion Server version 1.0.2 pipeline (15). Characteristics of the draft genome sequence of *L. braziliensis* IOC-L3564 are presented in Table 1.

Data availability. This draft genome sequence has been deposited at DDBJ/ENA/GenBank under the accession number cited in Table 1.

REFERENCES

1. Scott P, Novais FO. 2016. Cutaneous leishmaniasis: immune responses in protection and pathogenesis. Nat Rev Immunol 16:581–592. https://doi.org/10.1038/nri.2016.72.
2. Tirera S, Ginouves M, Donato D, Caballero IS, Bouchier C, Lavergne A, Bourreau E, Mosnier E, Vantilcke V, Couppié P, Prevot G, Lacoste V. 2017. Unraveling the genetic diversity and phylogeny of *Leishmania* RNA virus 1 strains of infected *Leishmania* isolates circulating in French Guiana. PLoS Negl Trop Dis 11:e0005764. https://doi.org/10.1371/journal.pntd.0005764.
3. Cantanhêde LM, Fernandes FG, Ferreira GEM, Porrozzi R, Ferreira RDGM, Cupolillo E. 2018. New insights into the genetic diversity of *Leishmania RNA virus 1* and its species-specific relationship with *Leishmania* parasites. PLoS One 13:e0198727. https://doi.org/10.1371/journal.pone.0198727.
4. Cupolillo E, Brahim LR, Toaldo CB, de Oliveira-Neto MP, de Brito MEF, Falqueto A, de Farias Naiff M, Grimaldi G. 2003. Genetic polymorphism and molecular epidemiology of *Leishmania* (*Viannia*) *braziliensis* from different hosts and geographic areas in Brazil. J Clin Microbiol 41:3126–3132. https://doi.org/10.1128/JCM.41.7.3126-3132.2003.
5. Kuhls K, Cupolillo E, Silva SO, Schweynoch C, Côrtes Boité M, Mello MN, Mauricio I, Miles M, Wirth T, Schönian G. 2013. Population structure and evidence for both clonality and recombination among Brazilian strains of the subgenus *Leishmania* (*Viannia*). PLoS Negl Trop Dis 7:e2490. https://doi.org/10.1371/journal.pntd.0002490.
6. Dumetz F, Imamura H, Sanders M, Seblova V, Myskova J, Pescher P, Vanaerschot M, Meehan CJ, Cuypers B, De Muylder G, Späth GF, Bussotti G, Vermeesch JR, Berriman M, Cotton JA, Volf P, Dujardin JC, Domagalska MA. 2017. Modulation of aneuploidy in *Leishmania donovani* during adaptation to different *in vitro* and *in vivo* environments and its impact on gene expression. mBio 8:e00599-17. https://doi.org/10.1128/mBio.00599-17.
7. Cupolillo E, Grimaldi G, Momen H. 1994. A general classification of new

world *Leishmania* using numerical zymotaxonomy. Am J Trop Med Hyg 50:296–311. https://doi.org/10.4269/ajtmh.1994.50.296.
8. Cantanhêde LM, da Silva Júnior CF, Ito MM, Felipin KP, Nicolete R, Salcedo JMV, Porrozzi R, Cupolillo E, Ferreira R. d G M. 2015. Further evidence of an association between the presence of *Leishmania RNA virus 1* and the mucosal manifestations in tegumentary leishmaniasis patients. PLoS Negl Trop Dis 9:e0004079. https://doi.org/10.1371/journal.pntd.0004079.
9. Aslett M, Aurrecoechea C, Berriman M, Brestelli J, Brunk BP, Carrington M, Depledge DP, Fischer S, Gajria B, Gao X, Gardner MJ, Gingle A, Grant G, Harb OS, Heiges M, Hertz-Fowler C, Houston R, Innamorato F, Iodice J, Kissinger JC, Kraemer E, Li W, Logan FJ, Miller JA, Mitra S, Myler PJ, Nayak V, Pennington C, Phan I, Pinney DF, Ramasamy G, Rogers MB, Roos DS, Ross C, Sivam D, Smith DF, Srinivasamoorthy G, Stoeckert CJ, Subramanian S, Thibodeau R, Tivey A, Treatman C, Velarde G, Wang H. 2010. TriTrypDB: a functional genomic resource for the *Trypanosomatidae*. Nucleic Acids Res 38:D457–D462. https://doi.org/10.1093/nar/gkp851.
10. 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.
11. Nurk S, Bankevich A, Antipov D, Gurevich A, Korobeynikov A, Lapidus A, Prjibelsky A, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, McLean J, Lasken R, Clingenpeel SR, Woyke T, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling genomes and mini-metagenomes from highly chimeric reads, p. 158–170. *In* RECOMB 2013: research in computational molecular biology. Springer, Berlin, Germany. https://doi.org/10.1007/978-3-642-37195-0_13.
12. Pryszcz LP, Gabaldón T. 2016. Redundans: an assembly pipeline for highly heterozygous genomes. Nucleic Acids Res 44:e113. https://doi.org/10.1093/nar/gkw294.
13. Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. 2009. ABACAS: algorithm-based automatic contiguation of assembled sequences. Bioinformatics 25:1968–1969. https://doi.org/10.1093/bioinformatics/btp347.
14. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
15. Steinbiss S, Silva-Franco F, Brunk B, Foth B, Hertz-Fowler C, Berriman M, Otto TD. 2016. *Companion*: a Web server for annotation and analysis of parasite genomes. Nucleic Acids Res 44:W29–W34. https://doi.org/10.1093/nar/gkw292.