



Draft Genome Sequence of *Trypanosoma equiperdum* Strain IVM-t1

Batdorj Davaasuren,^{a,b} Junya Yamagishi,^{c,d} Daiki Mizushima,^a Sandagdorj Narantsatsral,^b Davaajav Otgonsuren,^b Punsantsogvoo Myagmarsuren,^b Badgar Battsetseg,^b Banzragch Battur,^b Noboru Inoue,^e Keisuke Suganuma^{a,f}

^aNational Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

^bLaboratory of Molecular Genetics, Institute of Veterinary Medicine, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia

^cDepartment of Collaboration and Education, Research Center for Zoonosis Control, Hokkaido University, Sapporo, Hokkaido, Japan

^dGlobal Station for Zoonosis Control, GI-CoRE, Hokkaido University, Sapporo, Hokkaido, Japan

^eObihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

^fResearch Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

ABSTRACT *Trypanosoma equiperdum* primarily parasitizes the genital organs and causes dourine in equidae. We isolated a new *T. equiperdum* strain, *T. equiperdum* IVM-t1, from the urogenital tract of a horse definitively diagnosed as having dourine in Mongolia. Here, we report the whole-genome sequence, the predicted gene models, and their annotations.

Trypanosoma equiperdum belongs to the kingdom *Protista*, phylum *Sarcomastigophora*, class *Zoomastigophorea*, order *Kinetoplastida*, family *Trypanosomatidae*, genus *Trypanosoma*, subgenus *Trypanozoon* together with *T. brucei* and *T. evansi* (1). The taxonomy of *Trypanozoon* trypanosomes has been controversial because of their close evolutionary relationship and insufficient genetic markers. Despite the fact that many isolates taxonomically characterized as *T. equiperdum* were isolated over the past 50 years, it has been hypothesized that almost all of them likely represent misclassified *T. evansi* or *T. brucei* isolates (2). Recently, some *T. equiperdum* strains were newly isolated from Italy (strain ICT2011) (3), Ethiopia (strain Dodola) (4), and Venezuela (strains TeAp-N/D1 and TeGu-ND1) (5). Among the *Trypanozoon* group, whole-genome sequences of *T. brucei* strain TREU927, *T. evansi* strain STIB805, and *T. equiperdum* strain OVI were published in 2005 (6), 2015 (7), and 2017 (8), respectively. Here, we report the draft whole-genome sequence of the culture-adapted *T. equiperdum* strain IVM-t1, which was isolated from the urogenital tract of a stallion definitively diagnosed as having dourine in Mongolia (9).

The *T. equiperdum* strain IVM-t1 isolate was cultivated using Hirumi's modified Isocove's medium-9 (HMI-9) soft-agarose medium (HMI-9 with 0.8% low gelling agarose [type VII, Sigma-Aldrich Japan, Tokyo, Japan]) at 37°C in 5% CO₂ (9). The total DNA of culture-adapted *T. equiperdum* IVM-t1 was extracted and purified using Tris-EDTA (TE)-saturated phenol (Sigma-Aldrich Japan) and phenol-chloroform isoamyl-alcohol solution (Sigma-Aldrich Japan) (10). Purified DNA was kept at -30°C until use. The genome libraries of *T. equiperdum* IVM-t1 were prepared using a MiSeq reagent kit v3 for MiSeq sequencing (Illumina, Inc., CA), a DNA/polymerase binding kit P6 v2, and a DNA sequencing kit 4.0 v2 for PacBio RS II sequencing (Pacific Bioscience, Inc., CA). The MiSeq sequencer produced 28,337,050 paired-end reads with an average read length of 300 bp, and the PacBio RS II sequencer produced 7,508 reads with an average read length of 8,523 bp (range, 500 to 42,071 bp). The low-quality reads were trimmed using FastQC v0.11.5 and FASTX-Toolkit v0.0.13.

Whole-genome assembly was performed with ABySS-2.0.2 (11) with parameter

Citation Davaasuren B, Yamagishi J, Mizushima D, Narantsatsral S, Otgonsuren D, Myagmarsuren P, Battsetseg B, Battur B, Inoue N, Suganuma K. 2019. Draft genome sequence of *Trypanosoma equiperdum* strain IVM-t1. *Microbiol Resour Announc* 8:e01119-18. <https://doi.org/10.1128/MRA.01119-18>.

Editor Antonis Rokas, Vanderbilt University

Copyright © 2019 Davaasuren et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Keisuke Suganuma, k.suganuma@obihiro.ac.jp.

Received 11 September 2018

Accepted 30 January 2019

Published 28 February 2019

settings of $-pe$, $np = 12$, and $k = 68$ for MiSeq reads and with Hierarchical Genome Assembly Process 3 (HGAP.3) (12) at the default settings for PacBio reads. The MiSeq short reads were aligned to the contigs produced from PacBio reads using Bowtie v1.2.1.1 (13) with parameter settings of $-p 12$, $-x$, and $-S$. The quality of the contigs, which were derived from PacBio aligned with MiSeq, were improved using Pilon v1.21 (14) with parameter settings of $-Xmx128G$, $-changes$, $-vcf$, and $-tracks$. The obtained contigs were integrated into the draft genome of *T. equiperdum* by Metassembler v1.5 (15) with the parameter settings $bowtie2_maxins = 600$, $bowtie2_minins = 0$, and $meta2fasta_keepUnaligned = 0$. The chromosome construction, gene prediction, and gene annotation of each predicted gene of *T. equiperdum* IVM-t1 were performed using the published genome of *T. brucei brucei* (*T. b. brucei*) TREU927 as a reference via the Companion pipeline (<https://companion.sanger.ac.uk/>) (16) with default settings and without transcript evidence.

The integrated draft genome consisted of 45 contigs with an N_{50} value of 859,849 bp and a cumulative length of 26,988,997 bp (≈ 27 Mbp). The *T. equiperdum* IVM-t1 draft genome contains 7,718 protein-coding genes, 102 noncoding genes, and 2,473 pseudogenes. Following comparison of the predicted genes of *T. equiperdum* IVM-t1 with the reference gene sets of *T. b. brucei* TREU927 using OrthoMCL v2.0.7 (17), 6,831 protein-coding genes shared between the two species and 880 *T. equiperdum* IVM-t1-specific protein-coding genes were identified. Seven short genes that have less than 25 amino acids were not included in the comparison (<https://doi.org/10.6084/m9.figshare.7552262.v1>).

In conclusion, the whole-genome draft assembly produced in the present study provides a resource for future trypanosome genetic studies and identifies some *T. equiperdum*-specific genes.

Data availability. This whole-genome project has been deposited in DDBJ/ENA/GenBank under the accession no. [QSBY00000000](https://www.ncbi.nlm.nih.gov/nuccore/QSBY00000000). The version described in this paper is the first version, QSBY01000000. Raw sequence reads have been deposited in the NCBI SRA database under the accession no. [PRJNA477427](https://www.ncbi.nlm.nih.gov/sra/PRJNA477427).

ACKNOWLEDGMENTS

We thank Noriko Endo, Bat-Uyanga Lhagva, and Shoko Hayakawa for their project management in Mongolia. We thank all of the Mongolian SATREPS project researchers for supporting the present study.

This study was financially supported by the Japan Society for the Promotion of Science (JSPS), KAKENHI grant no. 16K18793 (Grants-in-Aid for Young Scientists [B]), Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and the AMED/JICA SATREPS (17jm0110006h0005).

REFERENCES

1. Stevens JR, Brisse S. 2004. The systematics of trypanosomes of medical and veterinary importance. In Maudlin I, Holmes PH, Miles MA (eds), *The trypanosomiasis*. CABI, Wallingford, UK.
2. Claes F, Buscher P, Touratier L, Goddeeris BM. 2005. *Trypanosoma equiperdum*: master of disguise or historical mistake? *Trends Parasitol* 21:316–321. <https://doi.org/10.1016/j.pt.2005.05.010>.
3. Pascucci I, Di Provido A, Camma C, Di Francesco G, Calistri P, Tittarelli M, Ferri N, Scacchia M, Caporale V. 2013. Diagnosis of dourine in outbreaks in Italy. *Vet Parasitol* 193:30–38. <https://doi.org/10.1016/j.vetpar.2012.12.006>.
4. Hagos A, Goddeeris B, Yilkal K, Alemu T, Fikru R, Yacob H, Feseha G, Claes F. 2010. Efficacy of Cymelarsan and Diminisan against *Trypanosoma equiperdum* infections in mice and horses. *Vet Parasitol* 171:200–206. <https://doi.org/10.1016/j.vetpar.2010.03.041>.
5. Sanchez E, Perrone T, Recchimuzzi G, Cardozo I, Biteau N, Aso PM, Mijares A, Baltz T, Berthier D, Balzano-Nogueira L, Gonzatti MI. 2015. Molecular characterization and classification of *Trypanosoma* spp. Venezuelan isolates based on microsatellite markers and kinetoplast maxicircle genes. *Parasit Vectors* 8:536. <https://doi.org/10.1186/s13071-015-1129-2>.
6. Berriman M, Ghedin E, Hertz-Fowler C, Blandin G, Renauld H, Bartholomeu DC, Lennard NJ, Caler E, Hamlin NE, Haas B, Böhme U, Hannick L, Aslett MA, Shallom J, Marcello L, Hou L, Wickstead B, Alsmark UCM, Arrowsmith C, Atkin RJ, Barron AJ, Bringaude F, Brooks K, Carrington M, Cherevach I, Chillingworth T-J, Churcher C, Clark LN, Corton CH, Cronin A, Davies RM, Doggett J, Djikeng A, Feldblyum T, Field MC, Fraser A, Goodhead I, Hance Z, Harper D, Harris BR, Hauser H, Hostetler J, Ivens A, Jagels K, Johnson D, Johnson J, Jones K, Kerhornou AX, Koo H, Larke N, et al. 2005. The genome of the African trypanosome *Trypanosoma brucei*. *Science* 309:416–422. <https://doi.org/10.1126/science.1112642>.
7. Carnes J, Anupama A, Balmer O, Jackson A, Lewis M, Brown R, Cestari I, Desquesnes M, Gendrin C, Hertz-Fowler C, Imamura H, Ivens A, Kořený L, Lai D-H, MacLeod A, McDermott SM, Merritt C, Monnerat S, Moon W, Myler P, Phan I, Ramasamy G, Sivam D, Lun Z-R, Lukeš J, Stuart K, Schnauffer A. 2015. Genome and phylogenetic analyses of *Trypanosoma evansi* reveal extensive similarity to *T. brucei* and multiple independent origins for dyskinetoplasty. *PLoS Negl Trop Dis* 9:e3404. <https://doi.org/10.1371/journal.pntd.0003404>.
8. Hebert L, Moumen B, Madeline A, Steinbiss S, Lakhdar L, Van Reet N, Buscher P, Laugier C, Cauchard J, Petry S. 2017. First draft genome

- sequence of the dourine causative agent: *Trypanosoma equiperdum* strain OVI. *J Genomics* 5:1–3. <https://doi.org/10.7150/jgen.17904>.
9. Suganuma K, Narantsatsral S, Battur B, Yamasaki S, Otgonsuren D, Musinguzi SP, Davaasuren B, Battsetseg B, Inoue N. 2016. Isolation, cultivation and molecular characterization of a new *Trypanosoma equiperdum* strain in Mongolia. *Parasit Vectors* 9:481. <https://doi.org/10.1186/s13071-016-1755-3>.
 10. Sambrook J, Russell DW, Sambrook J. 2006. *The condensed protocols from molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
 11. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. *Genome Res* 19:1117–1123. <https://doi.org/10.1101/gr.089532.108>.
 12. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Non-hybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
 13. Langmead B, Trapnell C, Pop M, Salzberg SL. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10:R25. <https://doi.org/10.1186/gb-2009-10-3-r25>.
 14. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
 15. Wences AH, Schatz MC. 2015. Metassembler: merging and optimizing de novo genome assemblies. *Genome Biol* 16:207. <https://doi.org/10.1186/s13059-015-0764-4>.
 16. 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>.
 17. Li L, Stoekert CJ, Jr, Roos DS. 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. *Genome Res* 13:2178–2189. <https://doi.org/10.1101/gr.1224503>.