Complete Genome Sequence of African Swine Fever Virus Isolated from a Domestic Pig in Timor-Leste, 2019

Patrick Mileto,a Felisiano da Conceição,b Vittoria Stevens,a David Cummins,a Andrea Certoma,a Matthew J. Neave,a Joanita Bendita da Costa Jong,b, and David T. Williamsa

aCSIRO, Australian Centre for Disease Preparedness, Geelong, Victoria, Australia
bNational Directorate of Veterinary Services of the Ministry of Agriculture and Fisheries, Government of Timor-Leste, Comoro, Dili, Timor-Leste

ABSTRACT Here, we report the complete genome sequence of the African swine fever virus (ASFV) isolate ASFV/Timor-Leste/2019/1, isolated from a domestic pig during the first outbreak of ASF in Timor-Leste in 2019. Using target enrichment short-read Illumina data combined with long-read Oxford Nanopore data, we assembled a full-length genome sequence of 192,237 bp.

African swine fever virus (ASFV) is a double-stranded DNA virus belonging to the genus *Asfivirus*, family *Asfarviridae*. It has a genome sequence approximately 170 to 194 kb long, encoding between 150 and 167 open reading frames. ASFV causes a devastating hemorrhagic disease in domestic and wild suids (1). Following its introduction into China in 2018, ASF spread rapidly through Southeast Asia (2) and emerged in Timor-Leste in September 2019 (3, 4).

Samples collected from sick and dying pigs in the Dili municipality were sent to the Australian Centre for Disease Preparedness for diagnostic testing and molecular characterization. An ASFV isolate was obtained from a nasal-rectal swab following inoculation onto primary porcine bone marrow cells and confirmed by ASFV PCR and specific immunofluorescence assay (5, 6). ASFV DNA was extracted from infectious tissue culture supernatant using the Applied Biosystems MagMAX-96 viral RNA kit (Thermo Fisher Scientific). A Nextera XT library (Illumina) was prepared using 2 ng of DNA extract, followed by ASFV-specific genome enrichment using the myBaits target capture kit (Arbor Biosciences), following the manufacturer’s instructions. The prepared library was then sequenced on an Illumina MiSeq instrument using the MiSeq reagent kit v.2 (150-bp paired-end [PE] reads).

To resolve the terminal repeats, the 3’ terminal repeat of the virus genome was amplified and the product sequenced using MinION long-read sequencing (Oxford Nanopore technology). The forward primer was designed to bind upstream of the 3’ terminal repeat (ASF_F_right: 5’-GTATTTACGGTAGGGTTTATTACCG), and the reverse primer bound slightly upstream of the terminal 3’ hairpin sequence (ASF_HP: 5’-GCAGGTACAATTTTATTATAGTGCAG). The region was amplified using the HotStarTaq master mix kit (Qiagen) with the following thermocycler conditions: 95°C for 15 min; 5 cycles of 95°C for 45 s, 40°C for 45 s, and 72°C for 2 min; 30 cycles of 95°C for 45 s, 55°C for 45 s, and 72°C for 2 min; and then a final extension of 72°C for 7 min. Oxford Nanopore libraries were prepared using the PCR barcoding kit (SQK-PBK004) and sequenced on an R9.4.1 flow cell (FLO-MIN106) for 48 h.

Default parameters were used for all software cited unless otherwise specified. For the genome assembly, the Illumina reads were mapped to the ASFV China/2018/AnhuiXCGQ genome (GenBank accession number MK128995.1) using the Geneious v.11.1.4 mapping algorithm and a draft consensus was generated. To complete the terminal repeats, the adapters were trimmed from the Oxford Nanopore reads using Porechop v.0.2.1 (7), and reads shorter than 1,360 bp and larger than 1,375 bp were removed using NanoFilt v.2.3.0 (8). The filtered reads were aligned using MAFFT v.7.407 (9) and imported into Geneious.
The ASFV/Timor-Leste/2019/1 genome contains 17 single nucleotide polymorphisms (SNPs) compared to China/2018/AnhuiXCGQ. Sequence analysis indicated that ASFV/Timor-Leste/2019/1 belongs to p72 genotype II, CD2v serogroup 8, IGR variant II, and CVR 1 subtype, characteristic of European, Chinese, and Southeast Asian viruses (10–13).

**Data availability.** This assembled genome sequence has been deposited in NCBI GenBank under the accession number MW396979. The raw sequence data have been deposited in the NCBI Sequence Read Archive (SRA) database under the BioProject accession number PRJNA725035.

**ACKNOWLEDGMENTS**

This work was supported by funding from the Australian Department of Agriculture, Water and the Environment (DAWE) and the European Commission Horizon 2020 Innovation and Research program (grant agreement number 871029-EVA-GLOBAL).

We gratefully acknowledge the DAWE officers involved in the specimen collection and shipment to ACDP. In addition, we acknowledge the contributions of the Timor-Leste National Directorate of Veterinary Team involved in the ASF outbreak response and the technical support of the Molecular Diagnostics Team at ACDP.

**REFERENCES**

1. Dixon LK, Stahl K, Jori F, Vial L, Pfiffer DU. 2020. African swine fever epidemiology and control. Annu Rev Anim Biosci 8:221–246. https://doi.org/10.1146/annurev-animal-021419-083741.
2. Zhou X, Li N, Luo Y, Liu Y, Miao F, Chen T, Zhang S, Cao P, Li X, Tian K, Qiu H-J, Hu R. 2018. Emergence of African swine fever in China, 2018. Transbound Emerg Dis 65:1482–1484. https://doi.org/10.1111/tbed.12989.
3. OIE. 2019. 27/09/2019: African swine fever, Timor-Leste (immediate notification), vol. 32.
4. Barnes TS, Morais O, Cargill C, Parke CR, Urlings A. 2020. First steps in managing the challenge of African swine fever in Timor-Leste. One Health 10:100151. https://doi.org/10.1016/j.jgiene.2020.100151.
5. Zsak L, Borca MV, Risatti GR, Zsak A, French RA, Lu Z, Kutish GF, Neilan JG, Callahan JD, Nelson WM, Rock DL. 2005. Preclinical diagnosis of African swine fever in contact-exposed swine by a real-time PCR assay. J Clin Microbiol 43:112–119. https://doi.org/10.1128/JCM.43.1.112-119.2005.
6. Jang C, Rowland RR, Allen JE, Cerotina A, Thissen JB, Birgham J, Rowe B, White JR, Wynne JW, Johnson D, Gaudreau NN, Williams DT. 2017. Gene expression analysis of whole blood RNA from pigs infected with low- and high-pathogenic African swine fever viruses. Sci Rep 7:10115. https://doi.org/10.1038/s41598-017-10186-4.
7. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Completing bacterial genome assemblies with multiplex MinION sequencing. Microb Genom 3:e000132. https://doi.org/10.1099/mgen.0.000132.
8. De Coster W, D’Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. Bioinformatics 34:2666–2669. https://doi.org/10.1093/bioinformatics/bty149.
9. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/msm010.
10. Gallardo C, Fernández-Pinero J, Pelayo V, Gazeau I, Markowska-Daniel I, Pridotkas G, Nieto R, Fernández-Pacheco P, Bokhan S, Nevolko O, Drozhzhe Z, Pérez C, Soler A, Kolvasov D, Arias M. 2014. Genetic variation among African swine fever genotype II viruses, Eastern and Central Europe. Emerg Infect Dis 20:1544–1547. https://doi.org/10.3201/eid2009.140554.
11. Ge S, Liu Y, Li L, Wang Q, Li J, Ren W, Liu C, Bao J, Wu X, Wang Z. 2019. An extra insertion of tandem repeat sequence in African swine fever virus, China, 2019. Virus Genes 55:843–847. https://doi.org/10.1007/s11262-019-01704-9.
12. Kim H-J, Cho K-H, Ryu J-H, Jang M-K, Chae H-G, Choi J-D, Nah J-J, Kim Y-J, Kang H-E. 2020. Isolation and genetic characterization of African swine fever virus from domestic pig farms in South Korea, 2019. Viruses 12:1237. https://doi.org/10.3390/v12111237.
13. Tran HTT, Truong AD, Dang AK, Ly DV, Nguyen CT, Chu NT, Nguyen HT, Dang HV. 2021. Genetic characterization of African swine fever viruses circulating in North Central region of Vietnam. Transbound Emerg Dis 68:1697–1699. https://doi.org/10.1111/tbed.13835.

---

### Table 1: Summary details of the ASFV/Timor-Leste/2019/1 genome sequence

| Isolate name         | Genome size (bp) | GC content (%) | No. of ORFs<sup>a</sup> | No. of Illumina reads | No. of mapped Illumina reads | No. of Oxford Nanopore reads | No. of mapped Oxford Nanopore reads | GenBank accession no. | BioProject accession no. |
|----------------------|------------------|----------------|------------------------|----------------------|-----------------------------|------------------------------|------------------------------------|----------------------|--------------------------|
| ASFV/Timor-Leste/2019/1 | 192,237          | 38.3           | 178                    | 226,084              | 111,527                     | 31,493                       | 5,152                              | MW396979             | PRJNA725035              |

<sup>a</sup>ORFs, open reading frames.