

Complete Genome Sequence of a Genomovirus Associated with Common Bean Plant Leaves in Brazil

Natalia Silva Lamas,^a Rafaela Salgado Fontenele,^a Fernando Lucas Melo,^b Antonio Felix Costa,^c Arvind Varsani,^{d,e} Simone Graça Ribeiro^a

Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil^a; Universidade de Brasília, Brasília, Brazil^b; Instituto Agronômico de Pernambuco, Recife, Pernambuco, Brazil^c; Structural Biology Research Unit, Department of Clinical Laboratory Sciences, University of Cape Town, Cape Town, South Africa^d; Center of Fundamental and Applied Microbiomics, The Bidesign Institute and School of Life Sciences, Arizona State University, Tempe, Arizona, USA^e

A new genomovirus has been identified in three common bean plants in Brazil. This virus has a circular genome of 2,220 nucleotides and 3 major open reading frames. It shares 80.7% genome-wide pairwise identity with a genomovirus recovered from Tongan fruit bat guano.

Received 17 September 2016 Accepted 21 September 2016 Published 10 November 2016

Citation Lamas NS, Fontenele RS, Melo FL, Costa AF, Varsani A, Ribeiro SG. 2016. Complete genome sequence of a genomovirus associated with common bean plant leaves in Brazil. *Genome Announc* 4(6):e01247-16. doi:10.1128/genomeA.01247-16.

Copyright © 2016 Lamas 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 Simone Graça Ribeiro, simone.ribeiro@embrapa.br.

Genomoviridae is a recently established family of circular replication-associated protein (Rep)-encoding single-stranded (CRESS) DNA viruses (1). The family currently has one genus, *Gemycircularvirus*, with a single recognized species, *Sclerotinia gemycircularvirus* 1. There are more than 100 genomoviruses that have been identified in various organisms and environmental samples summarized in Krupovic et al. (1). Within a Brazilian context, genomoviruses have been identified in human feces, river, and wastewater samples (2, 3). Here, we report the recovery of genomovirus genomes from bean plants (*Phaseolus vulgaris*) showing viral-like symptoms, such as leaf curling, mosaic, and stunting, which were collected in Arcoverde, Pernambuco state, Brazil.

Total DNA was extracted from these leaf samples, and circular viral DNA was enriched by rolling circle amplification (RCA) using Phi29 DNA polymerase (NEB, USA) (4). The RCA amplicons from all plants were pooled and sequenced using a GS-FLX + 454 sequencing platform at Macrogen Inc. (South Korea). The reads were analyzed for sequence similarities to CRESS DNA viral sequences. A 123-nucleotide (nt) read with similarities to genomovirus sequences was identified, and back-to-back primers (GemyNew forward primer 5' TCGAATCCATCGGGTATTGTGGG 3' and GemyNew reverse primer 5' ATTCGATACGGACGGCCTATATC 3') were designed to recover the full viral genomes from individual samples by inverse PCR with Phusion DNA polymerase (NEB). Amplicons of ~2.2 kb were obtained from three samples, which were gel-purified (GE Healthcare, USA), cloned into PCRII-TOPO-TA vector (Thermo Fisher Scientific Inc., USA) and Sanger-sequenced at Macrogen Inc. by primer walking. The complete circular genomes of 2,220 nt in length have three large bidirectionally oriented open reading frames (ORFs). One ORF in the virion sense encodes a putative viral capsid protein and, in the antisense, two other proteins are encoded, Rep and RepA. The

new sequences have been tentatively named common bean-associated gemycircularvirus (CBaGmV). The CBaGmV genome sequences share 80.7% genome-wide nucleotide pairwise identity with a genomovirus from Pacific flying fox feces (PfffaCV-6; accession no. KT732799). The CBaGmV CP, Rep, and RepA amino acid sequences are, respectively, 76%, 88%, and 88% similar to the homologous proteins of PfffaCV-6 (5).

Accession number(s). The complete genomes of CBaGmV described in this report have been deposited in GenBank under accession numbers [KX434768](https://www.ncbi.nlm.nih.gov/nuclseq/KX434768) to [KX434770](https://www.ncbi.nlm.nih.gov/nuclseq/KX434770).

ACKNOWLEDGMENTS

This research was sponsored by EMBRAPA, CNPq, and FAPDF. N.S.L. and R.S.F. are supported by a scholarship from CNPq.

REFERENCES

1. Krupovic M, Ghabrial SA, Jiang D, Varsani A. 2016. *Genomoviridae*: a new family of widespread single-stranded DNA viruses. *Arch Virol* 161:2633–2643. <http://dx.doi.org/10.1007/s00705-016-2943-3>.
2. Phan TG, Mori D, Deng X, Rajindrajith S, Ranawaka U, Fan Ng TF, Bucardo-Rivera F, Orlandi P, Ahmed K, Delwart E. 2015. Small circular single stranded DNA viral genomes in unexplained cases of human encephalitis, diarrhea, and in untreated sewage. *Virology* 482:98–104. <http://dx.doi.org/10.1016/j.virol.2015.03.011>.
3. Da Silva Assis MR, Vieira CB, Fioretti JM, Rocha MS, de Almeida PI, Miagostovich MP, Fumian TM. 2016. Detection and molecular characterization of gemycircularvirus from environmental samples in Brazil. *Food Environ Virol* [Epub ahead of print.] <http://dx.doi.org/10.1007/s12560-016-9254-4>.
4. Inoue-Nagata AK, Albuquerque LC, Rocha WB, Nagata T. 2004. A simple method for cloning the complete begomovirus genome using the bacteriophage ϕ 29 DNA polymerase. *J Virol Methods* 116:209–211. <http://dx.doi.org/10.1016/j.jviromet.2003.11.015>.
5. Male MF, Kraberger S, Stainton D, Kami V, Varsani A. 2016. Cycloviruses, gemycircularviruses and other novel replication-associated protein encoding circular viruses in Pacific flying fox (*Pteropus tonganus*) faeces. *Infect Genet Evol* 39:279–292. <http://dx.doi.org/10.1016/j.meegid.2016.02.009>.