

Changuinola Virus Serogroup, New Genomes within the Genus *Orbivirus* (Family *Reoviridae*) Isolated in the Brazilian Amazon Region

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We report here the first complete genome sequence of a *Changuinola virus* (CGLV) serotype *Irituia virus* (BE AN 28873) isolated from a wild rodent (*Oryzomys goeldi*) in the municipality of Ipixuna, State of Pará, northern Brazil. All genome segments showed similarity with those belonging to members of the genus *Orbivirus*, family *Reoviridae*.

Received 7 October 2013 Accepted 27 October 2013 Published 27 November 2013

Citation Silva SP, Dilcher M, Weidmann M, Carvalho VL, Casseb AR, Silva EVP, Nunes KNB, Chiang JO, Martins LC, Vasconcelos PFC, Nunes MRT. 2013. Changuinola virus serogroup, new genomes within the genus *Orbivirus* (family *Reoviridae*) isolated in the Brazilian Amazon region. *Genome Announc.* 1(6):e00940-13. doi:10.1128/genomeA.00940-13.

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The genus *Orbivirus* belongs to the family *Reoviridae* and includes 22 virus species, as well as 10 unclassified or unassigned viruses (1). Orbiviruses are classified based on their morphological, serological, and physicochemical characteristics (2, 3). They are icosahedral, nonenveloped viruses with double-stranded RNA (dsRNA). Their dsRNA genomes consist of 10 segments coding for 7 structural proteins (VP1 through VP7) and at least 3 nonstructural proteins (NS1 through NS3) (4). The serogroup Changuinola has 12 serotypes: *Changuinola virus*, *Almeirim virus*, *Altamira virus*, *Caninde virus*, *Gurupi virus*, *Irituia virus*, *Jamanxi virus*, *Jari virus*, *Monte Dourado virus*, *Ourem virus*, *Purus virus*, and *Saraca virus* (1).

Orbiviruses are transmitted between vertebrate hosts by a variety of arthropod vectors, including midges, ticks, phlebotomine flies, and anopheline and culicine mosquitoes (5). *Bluetongue virus* (BTV) and *African horse sickness virus* (AHSV) are the most economically important members of this genus. Several other orbiviruses are potentially important too, such as *Equine encephalosis virus* (EEV), *Epizootic hemorrhagic disease virus* (EHDV), *Palyam virus* (PALV), and *Peruvian horse sickness virus* (PHSV) (6), while other orbiviruses are causative agents of diseases in humans, such as *Kemerovo virus* (KEMV) and *Tribeč virus* (TRBV) (4).

The Brazilian CGLV serotype *Irituia virus* (BE AN 28873) was isolated at the Evandro Chagas Institute, Belém, Brazil, from the blood of a wild rodent species, *Oryzomys goeldi*, caught on 29 March 1961 in the municipality of Ipixuna, Brazil (3°S 49°W). Antigenic characterization was carried out by complement fixation and neutralization tests (3).

Brain tissue of newborn mice infected with serotype *Irituia virus* was propagated in Vero cells, and after 80% to 90% cytopathic effect (CPE) (3 days postinfection) the supernatant was harvested. Viral particles were concentrated using polyethylene glycol (PEG) centrifugation as previously described (4) and the

genomic dsRNA was isolated via a guanidinium isothiocyanate procedure (peqGold Trifast). After DNase treatment to digest cellular DNAs, viral dsRNA was purified from contaminating host single-stranded RNA (ssRNA) by overnight precipitation in 2 M lithium chloride at 4°C as described elsewhere (7).

Furthermore, a self-complementary full-length amplification of cDNAs (FLAC) adapter was ligated to the 3' ends of each RNA segment (8) to be able to cover the termini. The adapter-ligated dsRNA was converted to cDNA, amplified, and fragmented via the TransPlex whole-transcriptome amplification kit as previously described (4). A pyrosequencing library was prepared (9) and used for sequencing on a GS FLX 454 sequencer (Roche, Life Science) at the Department of Virology, University Medical Center, Göttingen, Germany. In parallel, the same library was sequenced in the Center for Technological Innovation at the Evandro Chagas Institute, Ministry of Health, Brazil.

The assembly of the obtained sequences was done using the GS *de novo* Assembler (Newbler v. 2.6). For further analysis, the software Geneious v. 6.1.4 and a set of programs (SeqMan Pro, SeqBuilder, and Protean) available in the DNASTAR package (Lasergene) were used. The coverage of the assembled genome was 1,522-fold. This is the first report for the complete genome sequence of a Brazilian CGLV serotype.

Nucleotide sequence accession numbers. The complete genome sequence has been deposited in GenBank under the accession numbers [KF624614](https://www.ncbi.nlm.nih.gov/nuccore/KF624614) to [KF624623](https://www.ncbi.nlm.nih.gov/nuccore/KF624623).

ACKNOWLEDGMENTS

This work was supported by the Evandro Chagas Institute (IEC), Ministry of Health, Coordination of Improvement of Higher Education Personnel (CAPES), and National Council of Technological and Scientific Development (CNPq) (process number 301641/2010-2 and grant number 302032/2011-8) in Brazil.

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