

# Emergence of Dengue Virus Serotype 2 Cosmopolitan Genotype, Brazil

## Appendix

### Materials and Methods

#### Ethics Statement

This project was reviewed and approved by the Pan American Health Organization Ethics Review Committee (reference no. PAHO-2016–08–0029) and Research Ethics Committee (Comitê de Ética em Pesquisa, CEP) of Federal University of Minas Gerais, Belo Horizonte, Brazil (CEP no. 32912820.6.1001.5149). Clinical samples are available for research purposes during outbreaks of national concern in accordance with the terms of the National Health Council Resolution no. 510/2016 and National Research Ethics Commission, Ministry of Health, Brazil (CONEP, Comissão Nacional de Ética em Pesquisa, Ministério da Saúde). This resolution authorizes the use of clinical samples collected in the Central Public Health Laboratory, Brazil, without informed consent to accelerate the acquisition of knowledge and contribute to surveillance and outbreak responses. The sample processed in this study was obtained anonymously from excess material after routine diagnosis of arboviruses by a public health laboratory that belongs to the public network within the Ministry of Health, Brazil.

#### Sample Collection, Viral RNA Isolation, and PCR Screening

A serum sample was collected for molecular diagnosis and sent to the Central Public Health Laboratory of Goiás for molecular screening. Viral RNA was extracted by using the QIAmp Viral RNA Mini Kit (QIAGEN, <https://www.qiagen.com>) and tested by quantitative reverse transcription PCR for arboviruses, including Zika virus, chikungunya virus, dengue virus (DENV) serotypes 1–4, and yellow fever virus. Molecular testing confirmed DENV-2 infection.

#### Library Preparation and Whole-Genome Sequencing

We prepared cDNA from viral RNA by using the ProtoScript II First Strand cDNA Synthesis Kit (*I*) and conducted multiplex tiling PCR by using Q5 High Fidelity Hot-Start DNA

Polymerase (New England Biolabs, <https://www.neb.com>) and DENV-2 sequencing primers (2,3). Amplicons were purified by using 1× AMPure XP Beads, and the PCR product concentrations were measured by using the Qubit dsDNA HS Assay Kit on a Qubit 3.0 fluorimeter (Thermo Fisher Scientific, <https://www.thermofisher.com>). We prepared a DNA library by using the Ligation Sequencing Kit and Native Barcoding Kit (EXP-NBD104; Oxford Nanopore Technologies, <https://nanoporetech.com>) (1). The DNA concentrations of purified PCR products were measured and normalized before the barcode reactions. The sequencing library was loaded onto a R9.4 flow cell (Oxford Nanopore), and data were collected for up to 6 h. In addition, this study included 2 complete genomes of the DENV-2 cosmopolitan genotype from the 2019 outbreak in Madre de Dios, Peru, that were sequenced by the US Centers for Disease Control and Prevention by using published tiling PCR methods on an Illumina MiSeq platform (GenBank accession nos. OM791800, OM791801, <https://www.ncbi.nlm.nih.gov/genbank>) (4,5).

#### **Generation of Consensus Sequences**

We performed base calling on raw files by using Guppy software and barcode demultiplexing by using qcat software. We generated de novo consensus sequence assemblies by using Genome Detective (<https://www.genomedetective.com>) (5). A total of 32,315 mapped reads were obtained with a mean sequencing depth of >1,000× and coverage of >86%. The DENV-2 cosmopolitan genotype was identified. The new DENV-2 sequence obtained in this study was deposited in GenBank (accession no. OM744143).

#### **Phylogenetic Analysis**

We constructed phylogenetic trees to explore the relationships between our sequenced DENV-2 genome and those of other isolated cosmopolitan genotypes. We retrieved 1,087 complete genomic coding sequences of DENV-2 cosmopolitan genotypes from GenBank; these data included the lineage date, country of collection, and the first 2 strains from South America isolated in Peru in 2019. Sequences were aligned by using MAFFT (6) and edited by using AliView (7). The datasets were assessed for the presence of phylogenetic signals by implementing the likelihood mapping analysis tool in the IQ-TREE 2 software (8). A maximum likelihood phylogeny was reconstructed by using IQ-TREE 2 software under the HKY+G4 substitution model (8). We inferred time-scaled trees by using TreeTime (9).

## References

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**Appendix Table.** List of accession numbers, collection dates, and locations for dengue virus serotype 2 cosmopolitan genotypes used to analyze genomic sequences in a study of outbreaks in Brazil\*

Accession no.	Location	Collection date	Accession no.	Location	Collection date
OM791800	Peru	2019-09-26	MK473386	Kenya	2016-01-15
OM791801	Peru	2019-09-28	MK513444	Singapore	2015-01-15
OM791802	Peru	2018-12-31	MK543448	China	2018-01-15
OM744143	Brazil	2021-11-29	MK543449	China	2018-01-15
AB189122	Indonesia	1998-01-15	MK543450	China	2018-01-15
AB189123	Indonesia	1998-01-15	MK543471	China	2018-01-15
AB189124	Indonesia	1998-01-15	MK543479	China	2018-01-15
AF276619	China	2000-01-15	MK564476	China	2017-01-15
AF359579	China	1999-01-15	MK564477	China	2016-01-15
AY037116	Australia	1993-01-15	MK564478	China	2016-01-15
AY776328	Taiwan	2004-01-15	MK564479	China	2016-01-15
AY858035	Indonesia	2004-01-15	MK564480	China	2016-01-15
AY858036	Indonesia	2004-01-15	MK564481	China	2017-01-15
DQ448231	India	2001-01-15	MK564482	China	2017-01-15
DQ645540	Taiwan	2001-10-31	MK564483	China	2017-01-15
DQ645541	Taiwan	2001-11-03	MK564484	China	2018-01-15
DQ645542	Taiwan	2001-11-12	MK564485	China	2018-01-15
DQ645543	Taiwan	2001-12-03	MK564486	China	2018-01-15
DQ645544	Taiwan	2001-12-07	MK564487	China	2018-01-15
DQ645545	Taiwan	2002-06-17	MK564488	China	2018-01-15
DQ645546	Taiwan	2002-06-24	MK578531	China	2016-01-15
DQ645547	Taiwan	2002-07-16	MK578532	China	2016-01-15
DQ645548	Taiwan	2002-07-20	MK578533	China	2018-01-15
DQ645549	Taiwan	2002-08-18	MK629884	South Korea	2015-01-15
DQ645550	Taiwan	2002-08-19	MK629885	South Korea	2015-01-15
DQ645551	Taiwan	2002-09-12	MK629886	South Korea	2015-01-15
DQ645552	Taiwan	2002-09-13	MK783189	China	2017-01-15
DQ645553	Taiwan	2002-10-23	MK783190	China	2018-01-15
DQ645554	Taiwan	2002-10-26	MK783191	China	2018-01-15
DQ645555	Taiwan	2002-11-01	MK783192	China	2018-01-15
DQ645556	Taiwan	2002-11-18	MK783193	China	2018-01-15
EF051521	China	2001-01-15	MK783194	China	2018-01-15
EU056810	Burkina Faso	1983-01-15	MK783195	China	2018-01-15
EU081177	Singapore	2005-01-15	MK783196	China	2018-01-15
EU081178	Singapore	2005-01-15	MK783197	China	2018-01-15
EU081179	Singapore	2005-01-15	MK783198	China	2018-01-15
EU081180	Singapore	2005-01-15	MK783199	China	2018-01-15
EU179857	Brunei	2005-01-15	MK783200	China	2017-01-15
EU179858	Brunei	2005-01-15	MK783201	China	2018-01-15
EU179859	Brunei	2006-01-15	MK783202	China	2018-01-15
EU359009	China	2007-01-15	MK783203	China	2018-01-15
EU482640	Viet Nam	2006-01-15	MK783204	China	2018-01-15
EU482672	Viet Nam	2006-01-15	MK783205	China	2018-01-15
FJ196852	China	2001-01-15	MK783206	China	2018-01-15
FJ196853	China	2003-01-15	MK783207	China	2018-01-15
FJ196854	China	1993-01-15	MK783208	China	2018-01-15
FJ882602	Sri Lanka	1996-01-15	MK783209	China	2018-01-15
FJ898454	India	2006-01-15	MK783210	China	2018-01-15
GQ252676	Sri Lanka	2003-01-15	MK858096	India	2014-01-15
GQ252677	Sri Lanka	2004-01-15	MK858097	India	2013-01-15
GQ398258	Indonesia	1975-01-15	MK858098	India	2016-01-15
GQ398259	Indonesia	1976-01-15	MK858099	India	2016-01-15
GQ398260	Indonesia	1976-01-15	MK858100	India	2016-01-15
GQ398261	Indonesia	1976-01-15	MK858101	India	2016-01-15
GQ398262	Indonesia	1976-01-15	MK858102	India	2016-01-15
GQ398263	Indonesia	1975-01-15	MK858103	India	2016-01-15
GQ398264	Indonesia	1976-01-15	MK858104	India	2016-01-15
GQ398265	Singapore	2008-01-15	MK858105	India	2016-01-15
GQ398266	Singapore	2007-01-15	MK858106	India	2016-01-15
GQ398267	Singapore	2007-01-15	MK858107	India	2016-01-15
GU131843	Burkina Faso	1986-01-15	MK858108	India	2016-01-15
GU370050	Singapore	2007-04-01	MK858109	India	2016-01-15
GU370051	Singapore	2008-08-01	MK858110	India	2016-01-15
HM488257	Guam	2001-01-15	MK858111	India	2016-01-15
JE963511	NULL	2015-01-15	MK858112	India	2017-01-15
JF327392	Singapore	2009-01-15	MK858113	India	2017-01-15
JN851113	Singapore	2006-01-15	MK858114	India	2017-01-15

Accession no.	Location	Collection date	Accession no.	Location	Collection date
JN851114	Singapore	2007-01-15	MK858115	India	2017-01-15
JN851115	Singapore	2007-01-15	MK858116	India	2017-01-15
JN851116	Singapore	2007-01-15	MN018337	China	2015-01-15
JN851117	Singapore	2007-01-15	MN018338	China	2015-01-15
JN851118	Singapore	2008-01-15	MN018339	China	2014-01-15
JN851119	Singapore	2008-01-15	MN018340	China	2016-01-15
JN851120	Singapore	2007-01-15	MN018341	China	2017-01-15
JN851121	Singapore	2008-01-15	MN018342	China	2017-01-15
JN851122	Singapore	2008-01-15	MN018343	China	2017-01-15
JN851123	Singapore	2004-01-15	MN018344	China	2017-01-15
JN851124	Singapore	2005-01-15	MN018346	China	2016-01-15
JN851125	Singapore	2005-01-15	MN018347	China	2016-01-15
JN851126	Singapore	2005-01-15	MN018348	China	2016-01-15
JN851127	Singapore	2004-01-15	MN018349	China	2015-01-15
JN851128	Singapore	2006-01-15	MN018350	China	2016-01-15
JN851129	Singapore	2006-01-15	MN018351	China	2015-01-15
JN851130	Singapore	2005-01-15	MN018352	China	2015-01-15
JN851131	Singapore	2005-01-15	MN018353	China	2016-01-15
JQ922549	India	1996-01-15	MN018354	China	2017-01-15
JQ922551	India	2005-01-15	MN018355	China	2017-01-15
JQ955623	India	2009-01-15	MN018356	China	2014-01-15
JQ955624	India	2011-01-15	MN018357	China	2014-01-15
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JX475906	India	2009-01-15	MN018359	China	2017-01-15
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KC762657	Indonesia	2008-04-23	MN018364	China	2016-01-15
KC762658	Indonesia	2007-08-14	MN018365	China	2015-01-15
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KC762661	Indonesia	2007-09-24	MN272405	Seychelles	2016-01-15
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KC762667	Indonesia	2008-04-12	MN335247	Kenya	2017-01-15
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KC762670	Indonesia	2007-12-11	MN566111	New Caledonia	2018-01-15
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KF041237	Pakistan	2009-01-15	MN577562	Kenya	2013-01-15
KF360005	Pakistan	2010-11-01	MN577563	Kenya	2013-01-15
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KF744397	Philippines	2001-01-15	MN577565	Kenya	2014-01-15
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KJ010186	Pakistan	2013-10-02	MN923108	China	2019-01-15
KJ701507	Pakistan	2013-08-27	MN923109	China	2019-01-15
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Accession no.	Location	Collection date	Accession no.	Location	Collection date
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KM279516	Singapore	2011-01-15	MN923121	China	2019-01-15
KM279517	Singapore	2011-01-15	MN944002	China	2019-01-15
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KM279526	Singapore	2011-01-15	MT006141	Sri Lanka	2017-01-15
KM279527	Singapore	2011-01-15	MT006142	Sri Lanka	2018-01-15
KM279528	Singapore	2012-01-15	MT006143	Sri Lanka	2018-01-15
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KM279532	Singapore	2012-01-15	MT006148	Sri Lanka	2017-01-15
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KM279565	Singapore	2012-01-15	MT261958	Burkina Faso	2017-01-15
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KM279567	Singapore	2012-01-15	MT261960	Burkina Faso	2017-01-15
KM279568	Singapore	2012-01-15	MT261961	Burkina Faso	2017-01-15
KM279569	Singapore	2012-01-15	MT261962	Burkina Faso	2017-01-15
KM279570	Singapore	2012-01-15	MT261963	Burkina Faso	2017-01-15
KM279571	Singapore	2012-01-15	MT261964	Burkina Faso	2017-01-15
KM279572	Singapore	2012-01-15	MT261965	Burkina Faso	2017-01-15
KM279573	Singapore	2012-01-15	MT261966	Burkina Faso	2017-01-15
KM279574	Singapore	2012-01-15	MT261967	Burkina Faso	2017-01-15
KM279575	Singapore	2012-01-15	MT261968	Burkina Faso	2017-01-15
KM279576	Singapore	2012-01-15	MT261969	Burkina Faso	2017-01-15
KM279577	Singapore	2012-01-15	MT261970	Burkina Faso	2017-01-15
KM279578	Singapore	2012-01-15	MT261971	Burkina Faso	2017-01-15
KM279579	Singapore	2012-01-15	MT754367	China	2019-01-15
KM279580	Singapore	2013-01-15	MT754368	China	2019-01-15

Accession no.	Location	Collection date	Accession no.	Location	Collection date
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KM279584	Singapore	2011-01-15	MT832053	Philippines	2014-01-15
KM279585	Singapore	2011-01-15	MT832054	Philippines	2014-01-15
KM279586	Singapore	2011-01-15	MT832055	Philippines	2013-01-15
KM279587	Singapore	2012-01-15	MT832056	Philippines	2013-01-15
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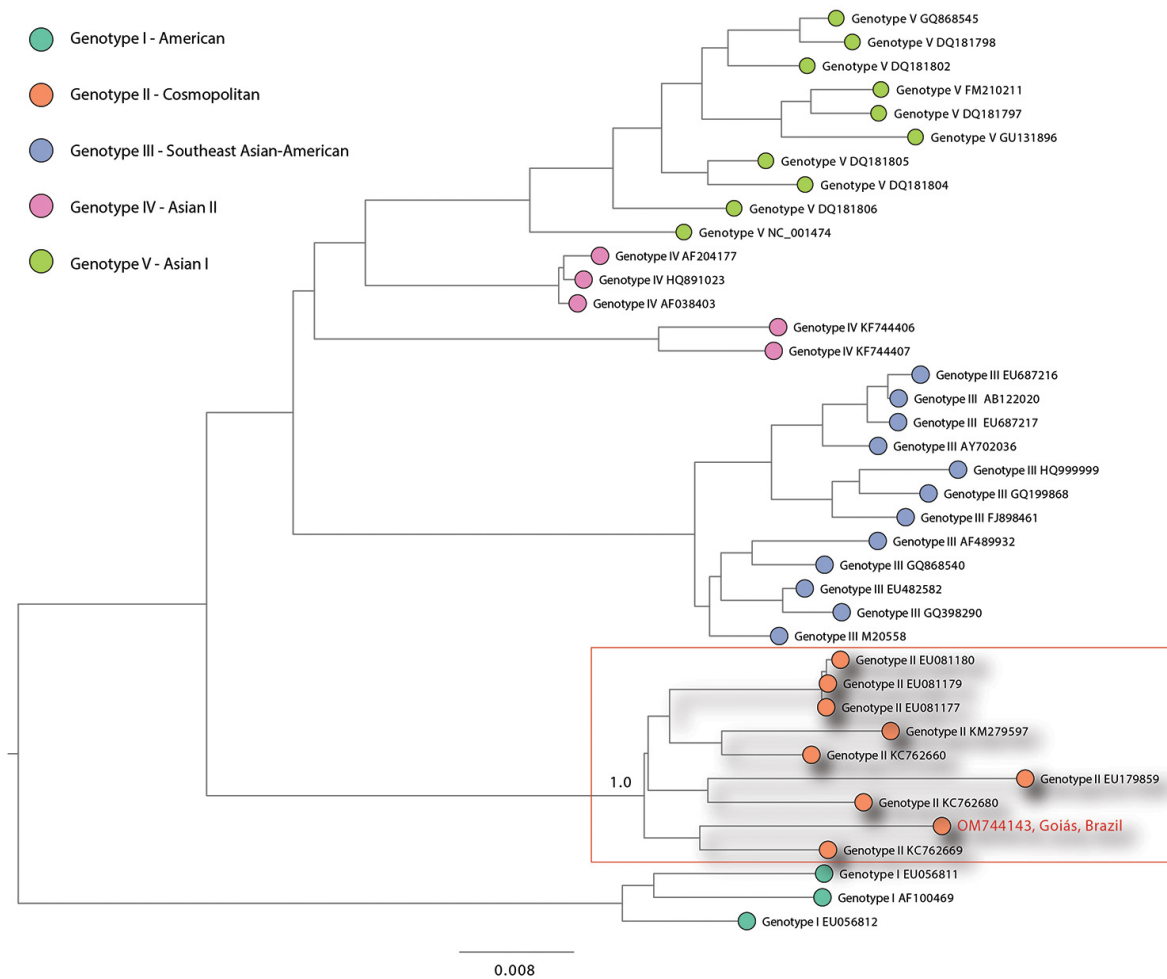


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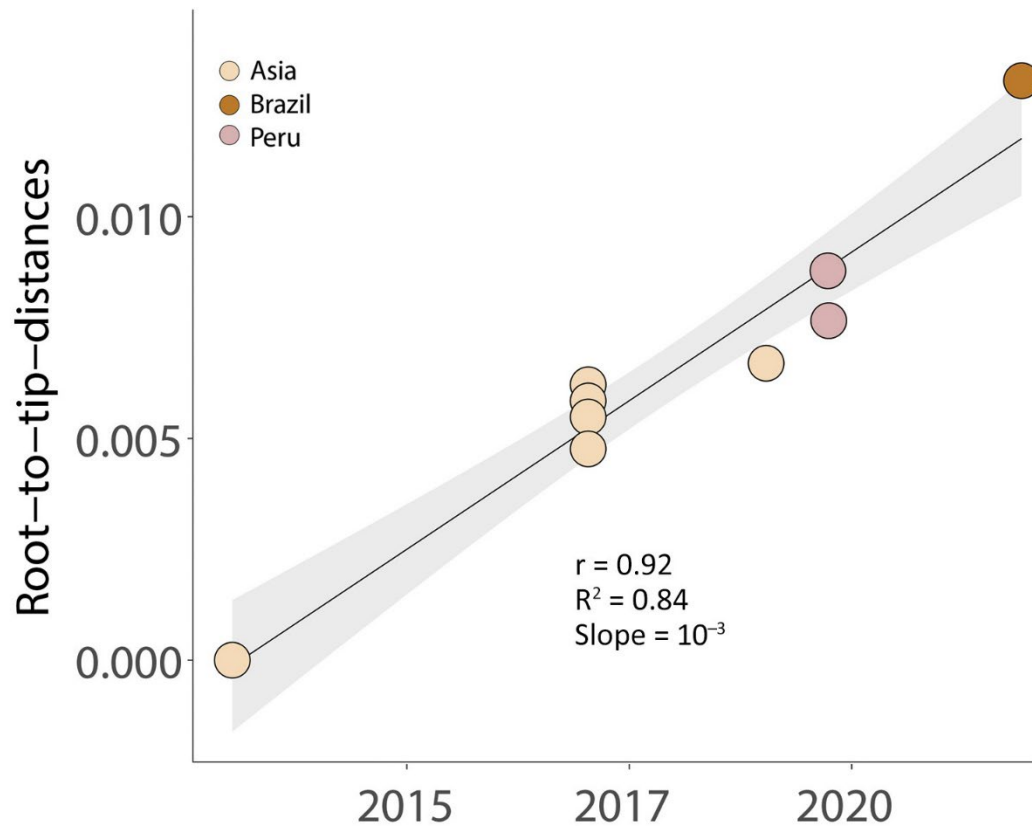
\*Accession numbers were obtained from GenBank.



**Appendix Figure 1.** Phylogenetic analysis of DENV-2 cosmopolitan genotype, Brazil. We used the maximum-likelihood method to compare the genome sequence generated in this study with 37 DENV-2 nonsylvatic genotypes retrieved from the National Center for Biotechnology Information database (<https://www.ncbi.nlm.nih.gov>). Red text indicates sequence described from Brazil. The nonsylvatic genotypes comprised DENV-2 Genotype I–American, DENV-2 Genotype II–cosmopolitan, DENV-2 Genotype III–Southern Asian-American, DENV-2 Genotype IV–Asian II, and DENV-2 Genotype V–Asian I. The boxed region indicates that the strain from Brazil clustered within a clade of cosmopolitan genotypes and had maximum statistical support (bootstrap value = 1.0). Scale bar indicates nucleotide substitutions per site. DENV-2, dengue virus serotype 2.



**Appendix Figure 2.** Map of sites in South America from which DENV-2 cosmopolitan genotype has been isolated. Gray area indicates Brazil; brown area indicates midwestern Brazil. Dark pink area indicates Peru. White circles indicate sampling locations of the DENV-2 cosmopolitan genotypes reported in Peru and Brazil. BR, Brazil; DENV-2, dengue virus serotype 2.



**Appendix Figure 3.** Root-to-tip regression plot of DENV-2 cosmopolitan genotype, Brazil. The root-to-tip genetic distances for genotypes presented in the boxed region of Appendix Figure 1 were plotted against sampling time. The cluster from South America diverged from strains observed in Bangladesh that were collected during 2017–2019, suggesting a complex transmission pattern mediated by transcontinental travel. DENV-2, dengue virus serotype 2.