Phylogenomics of Dengue Virus Isolates Causing Dengue Outbreak, São Tomé and Príncipe, 2022

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We determined that the dengue outbreak in São Tomé and Príncipe during 2022 was caused by dengue virus serotype 3 genotype III. Phylogenomic analyses showed that the outbreak strain was closely related to the newly identified GIII-American-II lineage and that the virus probably was introduced from the Americas.

Globally, dengue case numbers have increased dramatically over recent decades; an estimated 96 million clinical dengue cases per year have been reported in >100 countries (*1*). Dengue is an acute febrile disease that can evolve into a severe life-threatening disease. Dengue is caused by an infection with the dengue virus (DENV), a member of the family *Flaviviridae*, and has 4 different serotypes (DENV-1–4) and distinct infection dynamics (*2*).

In 2022, São Tomé and Príncipe, an island state with ≈210,000 inhabitants in the Gulf of Guinea in sub-Saharan Africa, reported the occurrence of dengue cases in the country. During epidemiologic weeks 15–50 in 2022, a total of 1,152 dengue fever cases confirmed by positive rapid diagnostic tests (RDTs) were reported. The first cases were reported April 15, and case numbers peaked at 178 notifications in week 24 (Appendix Figure, https://wwwnc.cdc.gov/EID/article/30/02/23-1316-App1.pdf). Among the 1,152 RDT-confirmed cases, the most frequent observed symptoms were fever (92%), headache (78%), and myalgia (38%). A total of 144 (12.5%) persons were admitted to the hospital (Appendix Table 1), and 8 persons died from infection with the virus. The presumptive index patient was described as a 27-year-old man from São Tomé and Príncipe who had traveled to the island of Guadeloupe before arriving in São Tomé on March 26, 2022, and whose onset of symptoms occurred on April 4, 2022 (*3*). A previous study analyzed the seroprevalence of DENV antibodies in the São Tomé and Príncipe population. In that study, 31 of 78 tested pregnant women were found to be seropositive for DENV, indicating that the country's population might have experienced exposure to the virus before 2003–2004, during which the collection of the analyzed serum samples took place (*4*).

This study was approved by the Health Ethics Committee for Scientific Research at the Ministry of Health of STP (approval no. 015B/2022). During May 6–16, 2022, we collected 7 plasma samples from dengue RDT–positive patients in São Tomé and Príncipe (Appendix Table 2). All 7 infections were confirmed by real-time PCR, and subtyping revealed the presence of DENV-3 (Appendix Table 2). Long-read whole-genome sequencing and subsequent assembly (reference strain GenBank accession no. NC_001475) resulted in 48–64,440 assembled reads (Appendix Table 3) with an average depth of coverage of 4–4,148× (Appendix Figure 2). We classified all 7 isolates as DENV-3 genotype III (GIII) by using a flavivirus genotyping tool (*5*) with bootstrap support of 100.

To study the evolutionary relationship of the virus isolates from São Tomé and Príncipe, we included 4 reconstructed genomes with best assembly results (>10 kb, genome coverage >98%, depth of coverage >250×) in a phylogenomic analysis together with 1,168 DENV-3 GIII genomes (Appendix Table 4) sampled worldwide. All 1,172 sequences passed the IQ-TREE2 composition test. The best-fitting evolutionary model according to Bayesian information criterion (BIC) was the general time-reversible plus empirical frequencies plus invariate sites plus FreeRate model. The reconstructed consensus tree revealed that the newly sequenced DENV-3 isolates from São Tomé and Príncipe clustered with and are closely related to the new monophyletic clade consisting of 218 DENV-3 sequences detected in the Americas during 2022–2023 (Figure). A recent study by Naveca et al. (*6*) demonstrates that this new lineage (GIII-American-II lineage) was most likely introduced to Cuba from the Indian subcontinent in

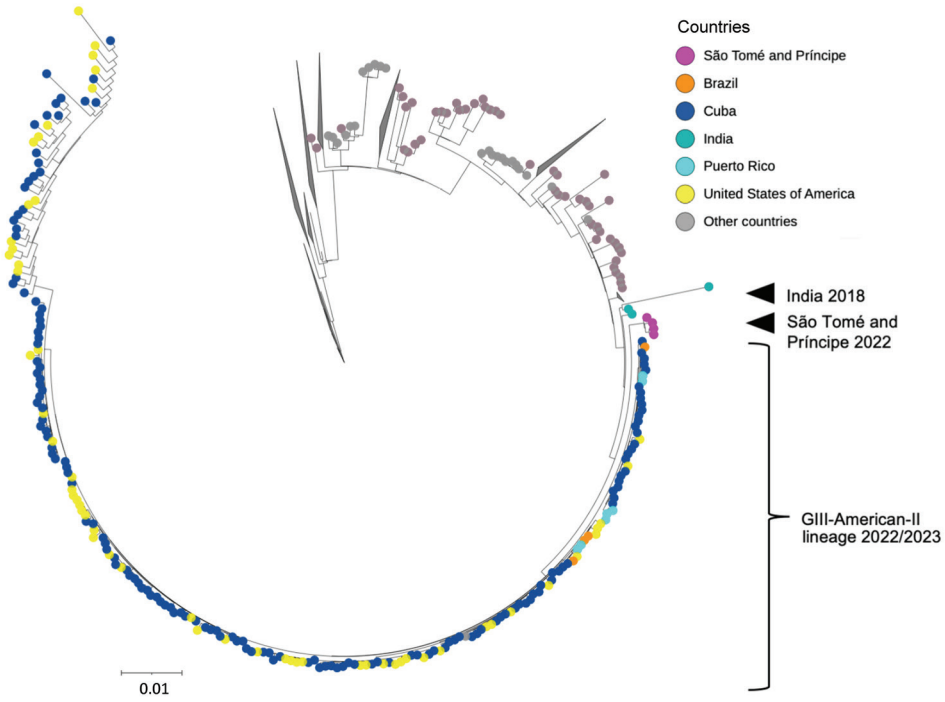

Figure. Reconstructed consensus tree of newly sequenced dengue virus serotype 3 isolates from São Tomé and Príncipe. The isolates clustered with and are closely related to a new monophyletic clade consisting of 218 dengue virus serotype 3 sequences detected in the Americas during 2022–2023. To improve visualization, several clades have been collapsed. Scale bar indicates nucleotide substitutions per site.

2019 (*6*). Consistent with their findings, our consensus tree (Figure) shows 3 DENV-3 sequences collected in India in 2018 as part of the next bigger clade comprising the GIII-American-II lineage and the 4 isolates from São Tomé and Príncipe.

Because the index patient reportedly had traveled to Guadeloupe before arriving in São Tomé and Príncipe, the likely scenario of virus importation is that after the introduction of the DENV-3-GIII lineage from Asia to America during 2018–2020, the virus might have circulated in the region, and from there it was introduced to São Tomé and Príncipe in 2022. Although we did not conduct formal phylogeographic analysis as part of this study, 2 points support our conclusions: the epidemiologic information that the index patient visited Guadalupe; and the results of the previous study from Brazil, describing the new DENV-3, GIII-American-II lineage and how it arose in America (*6*). Thus, our results suggest that the São Tomé and Príncipe outbreak originated from the new American lineage.

According to surveillance data of the Pan American Health Organization, Guadeloupe has experienced yearly dengue outbreaks since 2018, and in the year 2020, the serotypes 1–3 were detected (*7*). Unfortunately, no information is available on the DENV serotype or genomic sequences on the DENV circulating in 2022 in Guadeloupe, and only sparse information is available on dengue cases from countries in Africa.

The results of our study corroborate a possible global expansion of the new DENV-3 GIII-American-II clade previously described by Naveca et al. (*6*). Furthermore, finding this American lineage in Africa reinforces the importance of genomic surveillance of DENV in countries at risk for future outbreaks.

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We deposited the 4 dengue virus whole-genome sequences from this study in the European Nucleotide Archive at European Molecular Biology Laboratory–European Bioinformatics Institute (accession no. PRJEB65577).

Author contributions: L.L., D.W., K.T., A.B., A.G., A.S., M.N., N.T., Y.S.S., and A.K.L. performed the RNA extraction and the laboratory analyses; B.C.P and A.B.S. contributed the public health surveillance data; J.M. contributed to obtain funds; L.L., R.M.A.N., and K.S. designed the study and performed the bioinformatic analyses; and K.S. wrote the manuscript. All authors have read and approved the manuscript.

About the Author

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Integrating Veterinary Diagnostic Laboratories for Emergency Use Testing during Pandemics[1]

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The SARS-CoV-2 pandemic showed limitations in human outbreak testing. Veterinary diagnostic laboratories (VDLs) possess capabilities to bolster emergency test capacity. Surveys from 26 participating VDLs found human SARS-CoV-2 testing was mutually beneficial, including One Health benefits. VDLs indicated testing >3.8 million human samples during the pandemic, which included some challenges.

After emergence of SARS-CoV-2 in late January 2020, diagnostic testing was fraught with challenges. As cases increased, public health agencies struggled to provide timely support, prompting veterinary diagnostic laboratories (VDLs) to assist with processing human SARS-CoV-2 samples (*1*). VDLs regularly conduct diagnostic testing for infectious agents and maintain the necessary equipment, personnel, facilities, and protocols for animal disease testing. Currently, there are 60 university- or state-affiliated VDLs across the United States (*2*). On April 1, 2020, the World Organization for Animal Health published guidance stating that VDLs possess the resources and personnel expertise to help human diagnostic laboratories meet the demand for SARS-CoV-2 testing (*3*,*4*).

To assess VDL participation in human testing, we distributed a 14-question survey (Appendix, https://wwwnc.cdc.gov/EID/article/30/2/23-0562-App1.pdf) to 52 VDLs across the United States that had available email addresses. The study was reviewed by Colorado State University's Institutional Review Board (Protocol no. 3620), and respondent answers were deidentified before analysis. The first question queried whether human SARS-CoV-2 samples were tested and required an affirmative response to continue the survey. Subsequent questions were optional. Responses were gathered during July 7–December 22, 2022. Two follow-up reminders were sent during the open survey period. Responses were received from 26 (43.3%) of the 60 VDLs overall or 26 (50%) of the 52 VDLs that were contacted. Nine respondents indicated no human testing, and 17 (65.4%) of the 26 responding VDLs reported performing human testing. When >1 response was received from the same VDL (5 VDLs submitted >1 survey), numeric data were averaged, and all free text entries were included.

The duration of human testing across responding VDLs ranged from 5 to 31 months; average

[1]Preliminary results from this study were presented at the 65th American Association of Veterinary Laboratory Diagnosticians Conference and the 126th US Animal Health Association Annual Conference, October 6–12, 2022, Minneapolis, Minnesota, USA.

[2]These authors were co–principal investigators.

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Appendix

Material and Methods

Sample collection and RNA extraction

Seven plasma samples were collected randomly from patients that had an RDT positive test at the Hospital Dr. Ayres Menezes between 6th and 16th of May 2022 (Appendix Table 2). RNA was extracted from plasma using the QIAamp Viral RNA Mini Kit (Qiagen #52906) according to the manufacturer's instructions. Aliquots of the extracted nucleic acid were stored at −80°C until further analyses.

Real-time PCR and serotype identification

To detect DENV and identify the serotype real-time PCR was carried out using the RealStar Dengue RT-PCR Kit 3.0 (Altona Diagnostics #283003) and the RealStar Dengue Type RT-PCR Kit 1.0 (Altona Diagnostics #621003) according to manufacturer's instructions on a 7500 real-time PCR Instrument (Applied Biosystems).

Oxford Nanopore Technologies (ONT) library preparation

cDNA synthesis and multiplex PCR amplification were performed as previously described (*1*). Because the subtyping by real-time PCR did not reveal any evidence of infections with other serotypes than DENV-3, we used two pools of serotype-specific multiplex primer sets specifically designed for whole genome sequencing (WGS) of DENV-3 (*1*). Following PCR amplification, the two reactions were pooled and the final steps of the library preparation for the portable MinION sequencer (Oxford Nanopore Technologies) were performed as described (*2*).

Whole genome sequencing and consensus genomes

The sequencing run was performed on a MinION M1kc device (Oxford Nanopore Technology) and conversion from raw data to nucleotide sequences ("basecalling") in high-accuracy mode was performed with ONT Guppy basecalling software version 6.5.7. Fastq files for each of the seven DENV-3 isolates were uploaded into the Genome Detective Platform for default viral analyses and assembly of the consensus sequence based on DENV-3 reference sequence NC_001475 using the Genome Detective Virus Tool v2.12.2 (https://www.genomedetective.com) (*3*).

Genotype identification

For genotype identification based on the consensus sequences of the seven DENV-3 virus isolates we used the web application Flavivirus Genotyping Tool v0.1 (https://www.rivm.nl/mpf/typingtool/flavivirus/) (*4*).

DENV-3 dataset and phylogenomic reconstruction

The alignment and subsequent reconstruction of phylogenomic relationships of all publicly available DENV-3 genotype III genomes together with the four DENV-3 from STP was carried out as described previously (*5*). Before the analysis, the four STP sequences were visually inspected. None of them contained any undetermined nucleotide in the consensus sequence. All DENV-3 genotype III genomes with a genome length >10,000bp that were available from GenBank via the NCBI Virus portal were downloaded for the phylogenomic analyses. The search on the assessment day (30–06–2023) resulted in a total of 1,168 DENV-3 genomes (Appendix Table 3). Hence, the complete dataset used for alignment with MAFFT v7.520 (*6*) included 1,172 DENV3 genomes. The reconstruction of the phylogenomic relationships was done by maximum likelihood (ML) using IQ-TREE 2 multicore v2.2.2.6 (*7*). The best-fitting evolutionary model was selected. A ML phylogenetic tree was constructed using the best-fitting model based on Bayesian information criterion (BIC) tests with ModelFinder (*8*) and Ultrafast bootstrap with 2,000 replicates (*9*). Visualization of the best fitting consensus tree was done in iTOLv6 (*10*).

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Appendix Table 1. Age and sex distribution of 144 RDT-positive Dengue cases admitted to hospital during the 2022 Dengue outbreak in the Democratic Republic of São Tomé and Príncipe

Age interval [years]	No. of cases	Percentage [%]	Gender [M/F]
0–9	17	11.8	9/8
10–19	26	18.1	19/7
20–29	26	18.1	15/11
30–39	18	12.5	9/9
40–49	26	18.1	9/17
50–59	13	9.0	6/7
>60	18	12.5	5/13
Total	144	100.0	72/72

Appendix Table 2. Real-time PCR results for seven analyzed DENV-3 GIII isolates from the 2022 Dengue outbreak in the Democratic Republic of São Tomé and Príncipe*

ID	Collection date	Age [years]/ Gender	District	Ct	Serotype	Genotype
DENVSTP01	16.05.22	24/M	Água-Grande	24.9	3	III
DENVSTP02	06.05.22	40/M	Água-Grande	18.9	3	III
DENVSTP03	06.05.22	17/F	Água-Grande	28.3	3	III
DENVSTP04	12.05.22	8/F	Mezóchi	20.6	3	III
DENVSTP05	16.05.22	34/F	Água-Grande	20.6	3	III
DENVSTP06	16.05.22	25/F	Água-Grande	18.4	3	III
DENVSTP07	16.05.22	43/F	Água-Grande	16.1	3	III

*Ct, value of cycle threshold from real-time PCR

Appendix Table 3. Assembly statistics for seven sequenced DENV-3 GIII isolates from the 2022 Dengue outbreak in the Democratic Republic of São Tomé and Príncipe*

Sample ID	Ct value	#Reads	Coverage depth	NT Identity [%]	AA Identity [%]	Genome Coverage [%]	#Contigs	Genome length	ENA Accession number
DENVSTP01	24.9	3,460	169	95.7	98.5	91.5	3	9,798	–
DENVSTP02	18.9	51,915	2,302	95.7	98.4	97.9	1	10,484	ERS16303457
DENVSTP03	28.3	48	4	94.6	quality too low	45.9	10	4,911	–
DENVSTP04	20.6	1,108	71	95.6	98.3	66.2	8	7,083	–
DENVSTP05	20.6	5,166	259	95.7	98.6	97.7	1	10,461	ERS16303458
DENVSTP06	18.4	24,339	1,288	95.7	98.6	97.7	1	10,460	ERS16303459
DENVSTP07	16.1	64,440	4,148	95.8	98.7	98.3	1	10,526	ERS16303460

*The four in bold highlighted isolates were included in the phylogenomic analysis. Ct, real-time PCR cycle threshold; NT, nucleotide; AA, amino acid; Reference sequence used for assembly: NC_001475; ENA, European Nucleotide Archive.

Appendix Table 4. Publicly available DENV-3 GIII genomes used in the phylogenomic analysis (assessed on 30–06–2023)

Accession	Country	Collection date
AY099336	Sri Lanka	2000
AY099337	Martinique	1999
AY662691	Singapore	2004
AY679147	Brazil	2002
AY770511	India	2003
DQ675533	Taiwan	1999
EF629366	Brazil	2004–11
EF629367	Brazil	2004–11
EF629368	Brazil	2004–11
EF629369	Brazil	2002–01
EF643017	Brazil	2003
EU081181	Singapore	2004
EU081182	Singapore	2005
EU081183	Singapore	2005
EU081184	Singapore	2005
EU081185	Singapore	2005
EU081186	Singapore	2005
EU081187	Singapore	2005
EU081188	Singapore	2005
EU081189	Singapore	2005
EU081190	Singapore	2005
EU081191	Singapore	2005
EU081192	Singapore	2005
EU081193	Singapore	2005
EU081194	Singapore	2005
EU081195	Singapore	2005
EU081196	Singapore	2005
EU081197	Singapore	2005
EU081198	Singapore	2005
EU081199	Singapore	2005
EU081200	Singapore	2005
EU081201	Singapore	2005
EU081202	Singapore	2005
EU081203	Singapore	2005
EU081204	Singapore	2005
EU081205	Singapore	2005
EU081206	Singapore	2005
EU081207	Singapore	2005
EU081208	Singapore	2005
EU081209	Singapore	2005
EU081210	Singapore	2005
EU081211	Singapore	2005
EU081212	Singapore	2005
EU081213	Singapore	2005
EU081214	Singapore	2005
EU081215	Singapore	2005
EU081216	Singapore	2005
EU081217	Singapore	2005
EU081218	Singapore	2005
EU081219	Singapore	2005
EU081220	Singapore	2005
EU081222	Singapore	2005
EU081224	Singapore	2005
EU081225	Singapore	2005
EU482555	USA	2006
EU482558	USA	1998
EU482559	USA	1998
EU482563	USA	1998
EU482564	USA	2003
EU482566	USA	1998
EU482595	USA	2003
EU482596	USA	1998
EU482612	Venezuela	2001
EU482613	Venezuela	2001
EU482614	Venezuela	2001
EU529683	Venezuela	2007

Accession	Country	Collection date
EU529684	Venezuela	2001
EU529685	Venezuela	2001
EU529686	Venezuela	2001
EU529687	Venezuela	2001
EU529688	Venezuela	2001
EU529689	Venezuela	2001
EU529690	Venezuela	2001
EU529691	Venezuela	2001
EU529692	USA	2006
EU529696	USA	1999
EU529697	USA	2000
EU529698	USA	2006
EU529699	USA	2006
EU529702	USA	2003
EU529703	USA	1998
EU529704	USA	2004
EU529705	USA	2004
EU569688	Venezuela	2001
EU569689	Venezuela	2001
EU569690	Venezuela	2001
EU569691	Venezuela	2001
EU596492	USA	2007
EU596493	USA	2007
EU596494	USA	2007
EU660420	Venezuela	2001
EU687196	USA	2002
EU687197	USA	2003
EU687198	USA	2003
EU687218	USA	1998
EU687219	USA	1999
EU687221	USA	2000
EU687226	USA	1999
EU687233	USA	2002
EU687234	USA	2002
EU687239	USA	2003
EU726768	USA	2000
EU726769	USA	2003
EU726771	USA	1998
EU726772	USA	1998
EU726773	USA	1999
EU726774	USA	1999
EU781136	USA	1999
EU781137	USA	1999
EU854291	Venezuela	2004
EU854292	Venezuela	2005
EU854298	USA	2002
EU932687	Venezuela	2007
EU932688	Venezuela	2007
FJ024465	USA	2004
FJ024466	USA	2004
FJ024467	USA	2004
FJ024468	USA	2004
FJ024469	USA	2004
FJ024470	USA	2004
FJ024471	USA	2004
FJ177308	Brazil	2001
FJ182004	USA	2004
FJ182005	USA	2004
FJ182006	USA	2004
FJ182007	USA	2005
FJ182008	USA	2005
FJ182009	USA	2005
FJ182010	USA	2005
FJ182011	USA	2005
FJ182013	USA	1998
FJ182015	Venezuela	2001
FJ182037	USA	2005
FJ182038	USA	2005
FJ182039	USA	2005

Accession	Country	Collection date
FJ182040	USA	2005
FJ182041	USA	2005
FJ205870	USA	2003
FJ205871	USA	1999
FJ373302	USA	2004
FJ373303	Venezuela	2001
FJ373304	Venezuela	2004
FJ373306	USA	2002
FJ390371	USA	2003
FJ390372	USA	2003
FJ390373	USA	2002
FJ390375	USA	1999
FJ390376	USA	1999
FJ390377	USA	1999
FJ410176	USA	2000
FJ410177	USA	2000
FJ410178	USA	2002
FJ478456	USA	2002
FJ547069	USA	1999
FJ547070	USA	1998
FJ547071	USA	2000
FJ547072	USA	2000
FJ547073	USA	2000
FJ547074	USA	2000
FJ547075	USA	2000
FJ547076	USA	2000
FJ547077	USA	2000
FJ547078	USA	2000
FJ547079	USA	2001
FJ547080	USA	2001
FJ547081	USA	2001
FJ547082	USA	2001
FJ547083	USA	2002
FJ547084	USA	2002
FJ547085	USA	2006
FJ562107	USA	2000
FJ639746	Venezuela	2000
FJ639747	Venezuela	2000
FJ639749	Venezuela	2000
FJ639750	Venezuela	2000
FJ639751	Venezuela	2001
FJ639752	Venezuela	2001
FJ639753	Venezuela	2001
FJ639754	Venezuela	2001
FJ639755	Venezuela	2001
FJ639756	Venezuela	2001
FJ639757	Venezuela	2001
FJ639758	Venezuela	2001
FJ639759	Venezuela	2001
FJ639760	Venezuela	2001
FJ639761	Venezuela	2001
FJ639762	Venezuela	2001
FJ639763	Venezuela	2001
FJ639765	Venezuela	2001
FJ639766	Venezuela	2001
FJ639767	Venezuela	2001
FJ639768	Venezuela	2001
FJ639769	Venezuela	2001
FJ639770	Venezuela	2001
FJ639771	Venezuela	2001
FJ639772	Venezuela	2007
FJ639774	Venezuela	2001
FJ639775	Venezuela	2002
FJ639776	Venezuela	2002
FJ639777	Venezuela	2002
FJ639778	Venezuela	2002
FJ639779	Venezuela	2003
FJ639780	Venezuela	2003
FJ639781	Venezuela	2003

Accession	Country	Collection date
FJ639782	Venezuela	2003
FJ639784	Venezuela	2003
FJ639785	Venezuela	2003
FJ639786	Venezuela	2003
FJ639787	Venezuela	2004
FJ639789	Venezuela	2004
FJ639790	Venezuela	2004
FJ639791	Venezuela	2004
FJ639792	Venezuela	2004
FJ639793	Venezuela	2004
FJ639795	Venezuela	2004
FJ639798	Venezuela	2004
FJ639799	Venezuela	2004
FJ639800	Venezuela	2004
FJ639801	Venezuela	2004
FJ639803	Venezuela	2005
FJ639804	Venezuela	2005
FJ639805	Venezuela	2005
FJ639807	Venezuela	2005
FJ639810	Venezuela	2005
FJ639816	Venezuela	2005
FJ639817	Venezuela	2006
FJ639825	Venezuela	2006
FJ639826	Venezuela	2008
FJ639827	Venezuela	2008
FJ644564	India	2007
FJ744700	Venezuela	2001
FJ810416	Venezuela	2001
FJ850048	Nicaragua	2008
FJ850049	Nicaragua	2008
FJ850052	Nicaragua	2008
FJ850055	USA	2004
FJ850056	USA	2004
FJ850079	Brazil	2003
FJ850080	Brazil	2003
FJ850083	Brazil	2004
FJ850086	Brazil	2005
FJ850089	Brazil	2006
FJ850092	Brazil	2007
FJ850094	Brazil	2008
FJ850096	Venezuela	2001
FJ850097	Venezuela	2001
FJ850098	Venezuela	2001
FJ850109	Venezuela	2007
FJ850110	Venezuela	2007
FJ850111	Venezuela	2007
FJ873812	Nicaragua	2008
FJ873813	Nicaragua	2008
FJ882571	Sri Lanka	1989
FJ882572	Sri Lanka	1989
FJ882573	Sri Lanka	1993
FJ882574	Sri Lanka	1985
FJ882575	Mozambique	1985
FJ882576	Nicaragua	1994
FJ882577	Venezuela	2001
FJ882578	Venezuela	2001
FJ898440	Mexico	2003
FJ898441	Mexico	2006
FJ898442	Mexico	2007
FJ898443	Colombia	2003
FJ898444	Colombia	2005
FJ898445	Colombia	2007
FJ898446	Brazil	2001
FJ898447	Brazil	2003
FJ898457	Ecuador	2000
FJ898458	Peru	2002
FJ898459	Trinidad and Tobago	2002
FJ898462	Anguilla	2001
FJ898463	Saint Lucia	2001

Accession	Country	Collection date
FJ898464	Guyana	2002
FJ898468	Venezuela	2000
FJ898469	Venezuela	2001
FJ898470	Venezuela	2001
FJ898471	Venezuela	2002
FJ898472	Venezuela	2003
FJ898473	Venezuela	2003
FJ898474	Venezuela	2007
FJ898475	Nicaragua	2008
FJ898476	Nicaragua	2008
FJ913015	Brazil	2001
GQ199860	Nicaragua	2008
GQ199861	Nicaragua	2008
GQ199862	Nicaragua	2008
GQ199863	Nicaragua	2008
GQ199864	Nicaragua	2008
GQ199865	Nicaragua	2009
GQ199870	Nicaragua	2008
GQ199871	Nicaragua	2008
GQ199886	Nicaragua	1998
GQ199887	Sri Lanka	1983
GQ199888	Sri Lanka	1983
GQ199889	Sri Lanka	1983
GQ199891	Colombia	2001
GQ252674	Sri Lanka	1997
GQ252678	Venezuela	2001
GQ466079	India	2008
GQ868546	Brazil	2006
GQ868547	Brazil	2006
GQ868548	Brazil	2006
GQ868571	Colombia	2002
GQ868572	Colombia	2003
GQ868573	Colombia	2003
GQ868574	Colombia	2003
GQ868575	Colombia	2004
GQ868576	Colombia	2005
GQ868577	Colombia	2005
GQ868578	Colombia	2007
GQ868586	Venezuela	2007
GQ868587	Venezuela	2007
GQ868616	Saint Lucia	2001
GQ868617	Trinidad and Tobago	2002
GU131844	Brazil	2006
GU131845	Brazil	2006
GU131846	Brazil	2006
GU131847	Brazil	2006
GU131848	Brazil	2006
GU131849	Brazil	2006
GU131850	Brazil	2006
GU131851	Brazil	2006
GU131852	Brazil	2006
GU131853	Brazil	2006
GU131854	Brazil	2006
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GU131856	Brazil	2006
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GU131858	Brazil	2006
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GU131860	Brazil	2006
GU131861	Brazil	2007
GU131862	Brazil	2007
GU131865	Brazil	2006
GU131866	Brazil	2007
GU131867	Brazil	2007
GU131868	Brazil	2007
GU131869	Brazil	2007
GU131870	Brazil	2007
GU131871	Brazil	2007
GU131872	Brazil	2007

Accession	Country	Collection date
GU131873	Brazil	2007
GU131874	Brazil	2007
GU131875	Brazil	2007
GU131876	Brazil	2007
GU131877	Brazil	2007
GU131878	Brazil	2007
GU131950	Colombia	2001
GU131951	Colombia	2003
GU131952	Colombia	2003
GU131953	Colombia	2004
GU131954	Colombia	2006
GU363549	China	2009–08–06
GU370053	Singapore	2007–05
HM181972	Nicaragua	2009
HM181973	Nicaragua	2009
HM181974	Nicaragua	2009
HM181975	Nicaragua	2009
HM181976	Nicaragua	2009
HM181977	Nicaragua	2009
HM181978	Nicaragua	2009
HM631856	Nicaragua	2009
HM631857	Nicaragua	2009
HM631858	Nicaragua	2009
HM631859	Nicaragua	2009
HM631860	Nicaragua	2009
HM631861	Nicaragua	2009
HM631862	Nicaragua	2009
HM631863	Nicaragua	2009
HM631864	Nicaragua	2009
HM631869	Nicaragua	2009
HM756274	Nicaragua	2009
HM756275	Nicaragua	2009
HM756276	Nicaragua	2009
HM756277	Nicaragua	2009
HM756278	Nicaragua	2009
HM756279	Nicaragua	2009
HM756280	Nicaragua	2009
HM756281	Nicaragua	2009
HM756282	Nicaragua	2009
HQ166030	Nicaragua	2009
HQ166031	Nicaragua	2009
HQ166032	Nicaragua	2009
HQ166033	Nicaragua	2009
HQ166034	Nicaragua	2009
HQ235027	Paraguay	2007
HQ332170	Venezuela	2006
HQ332171	Venezuela	2006
HQ541785	Nicaragua	2009
HQ541789	Nicaragua	2008
HQ541790	Nicaragua	2008
HQ541791	Nicaragua	2008
HQ541795	Nicaragua	2009
HQ541796	Nicaragua	2009
HQ541797	Nicaragua	2009
HQ541802	Nicaragua	2009
HQ541804	Nicaragua	2009
HQ541806	Nicaragua	2009
HQ671176	Nicaragua	2009
HQ671177	Nicaragua	2009
HQ671178	Nicaragua	2009
HQ705609	Nicaragua	2009
HQ705610	Nicaragua	2009
HQ705611	Nicaragua	2009
HQ705612	Nicaragua	2009
HQ705613	Nicaragua	2009
HQ705614	Nicaragua	2009
HQ705615	Nicaragua	2009
HQ705616	Nicaragua	2009
HQ705617	Nicaragua	2009

Accession	Country	Collection date
HQ705618	Nicaragua	2009
HQ705619	Nicaragua	2009
HQ705620	Nicaragua	2009
HQ705621	Nicaragua	2009
HQ705622	Nicaragua	2009
HQ705623	Nicaragua	2009
HQ891025	Nicaragua	2009
JF504679	China	2009–09
JF808118	Brazil	2002
JF808119	Brazil	2004
JF808120	Brazil	2009
JF808121	Brazil	2007
JF808122	Paraguay	2003
JF808123	Paraguay	2002
JF808124	Brazil	2003
JF808125	Brazil	2002
JF808126	Brazil	2003
JF808127	Brazil	2002
JF808128	Paraguay	2003
JF808129	Paraguay	2003
JF920393	Nicaragua	2009
JF920394	Nicaragua	2009
JF920395	Nicaragua	2009
JF920396	Nicaragua	2009
JF920397	Nicaragua	2010
JF920398	Nicaragua	2009
JF920399	Nicaragua	2009
JF920400	Nicaragua	2009
JF920401	Nicaragua	2010
JF920402	Nicaragua	2009
JF920403	Nicaragua	2009
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JF920405	Nicaragua	2010
JF920406	Nicaragua	2010
JF920407	Nicaragua	2009
JF920408	Nicaragua	2010
JF920409	Nicaragua	2010
JF937620	Nicaragua	2010
JF937621	Nicaragua	2010
JF937622	Nicaragua	2010
JF937623	Nicaragua	2010
JF937624	Nicaragua	2010
JF937625	Nicaragua	2010
JF937626	Nicaragua	2010
JF937627	Nicaragua	2010
JF937628	Nicaragua	2010
JF937629	Nicaragua	2010
JF937630	Nicaragua	2009
JF937631	Nicaragua	2009
JF937632	Nicaragua	2009
JF937633	Nicaragua	2010
JF937634	Nicaragua	2010
JF937636	Nicaragua	2009
JF937637	Nicaragua	2010
JF937638	Nicaragua	2010
JF937639	Nicaragua	2010
JF937640	Nicaragua	2010
JF937641	Nicaragua	2009
JF937642	Nicaragua	2009
JF937643	Nicaragua	2009
JF937646	Nicaragua	2010
JF937647	Nicaragua	2009
JF937648	Nicaragua	2010
JF937652	Nicaragua	2009
JN000936	Nicaragua	2009
JN000937	Nicaragua	2010
JN000938	Nicaragua	2010
JN093513	Nicaragua	2010
JN093514	Nicaragua	2009

Accession	Country	Collection date
JN093515	Nicaragua	2010
JN093517	Nicaragua	2009
JN183884	Nicaragua	2009
JN662391	China	2009–08–06
JQ411814	Sri Lanka	1989
JQ922555	India	1966
JQ922556	India	2005
JQ922557	India	2005
JX669489	Brazil	2003
JX669490	Brazil	2002
JX669491	Brazil	2002
JX669492	Brazil	2005
JX669493	Brazil	2005
JX669494	Brazil	2005
JX669495	Brazil	2004
JX669496	Brazil	2006
JX669497	Brazil	2005
JX669498	Brazil	2004
JX669499	Brazil	2004
JX669500	Brazil	2005
JX669501	Brazil	2005
JX669502	Brazil	2005
JX669503	Brazil	2005
JX669504	Brazil	2006
JX669505	Brazil	2006
JX669506	Brazil	2006
JX669507	Brazil	2006
JX669508	Brazil	2006
KC425219	Brazil	2002
KF041254	Pakistan	2008
KF041255	Pakistan	2007
KF041256	Pakistan	2006
KF041257	Pakistan	2006
KF041258	Pakistan	2009
KF041259	Pakistan	2006
KF921913	Nicaragua	2009
KF921914	Nicaragua	2009
KF921916	Nicaragua	2009
KF921920	Nicaragua	2009
KF921921	Nicaragua	2009
KF921922	Nicaragua	2009
KF921923	Nicaragua	2009
KF921924	Nicaragua	2010
KF921926	Nicaragua	2010
KF921928	Nicaragua	2010
KF921929	Nicaragua	2010
KF954945	China	2013–08–08
KF954946	China	2013–08–08
KF954947	China	2013–08–08
KF954948	China	2013–08–08
KF954949	China	2013–08–08
KF955449	Venezuela	2001–10–13
KF955451	Venezuela	2003–12–01
KF955453	Venezuela	2003–12–19
KF955454	Venezuela	2004–11–07
KF955456	Puerto Rico	2006
KF955465	Puerto Rico	2000
KF955466	Puerto Rico	2000
KF955468	Puerto Rico	2001
KF955471	Venezuela	2004
KF955472	Venezuela	2004
KF955473	Brazil	2002
KF955474	Sri Lanka	1989
KF955479	Venezuela	2001
KF955481	Venezuela	2007
KF955486	Venezuela	2001
KF955487	Venezuela	2001
KF955490	Nicaragua	2008
KF955505	Grenada	2002

Accession	Country	Collection date
KF971695	Nicaragua	2009
KF971709	Nicaragua	2009
KF973476	Nicaragua	2011
KF973477	Nicaragua	2011
KF973478	Nicaragua	2012
KF973479	Nicaragua	2012
KF973480	Nicaragua	2012
KF973481	Nicaragua	2011
KF973482	Nicaragua	2011
KF973483	Nicaragua	2011
KF973484	Nicaragua	2011
KF973485	Nicaragua	2011
KF973486	Nicaragua	2012
KF973487	Nicaragua	2011
KJ189255	Peru	2002
KJ189256	Peru	2002
KJ189257	Peru	2004
KJ189258	Peru	2002
KJ189259	Peru	2002
KJ189260	Peru	2002
KJ189261	Peru	2008
KJ189262	Peru	2004
KJ189263	Peru	2004
KJ189264	Peru	2004
KJ189265	Peru	2004
KJ189266	Peru	2004
KJ189267	Peru	2007
KJ189268	Peru	2007
KJ189269	Peru	2007
KJ189270	Peru	2007
KJ189271	Peru	2007
KJ189272	Peru	2007
KJ189273	Peru	2007
KJ189274	Peru	2007
KJ189275	Peru	2007
KJ189276	Peru	2007
KJ189277	Peru	2007
KJ189278	Peru	2007
KJ189279	Peru	2007
KJ189280	Peru	2007
KJ189281	Peru	2007
KJ189282	Peru	2007
KJ189283	Peru	2007
KJ189284	Peru	2008
KJ189285	Peru	2008
KJ189286	Peru	2008
KJ189287	Peru	2008
KJ189288	Peru	2008
KJ189289	Peru	2008
KJ189290	Peru	2008
KJ189291	Peru	2008
KJ189292	Peru	2009
KJ189293	Peru	2005
KJ189294	Peru	2006
KJ189295	Peru	2006
KJ189296	Peru	2006
KJ189297	Peru	2006
KJ189298	Peru	2008
KJ189299	Peru	2005
KJ189300	Peru	2008
KJ189301	Peru	2008
KJ643590	Peru	2007
KJ830751	Saudi Arabia	2014–01–26
KT726340	Cuba	2001
KT726341	Cuba	2001
KT726342	Cuba	2001
KT726343	Cuba	2001
KT726344	Cuba	2001
KT726345	Cuba	2002

Accession	Country	Collection date
KT726346	Cuba	2002
KT726347	Cuba	2002
KT726348	Cuba	2002
KT726349	Cuba	2001
KT726350	Cuba	2001
KT726351	Cuba	2001
KT726352	Cuba	2001
KT726353	Cuba	2001
KT726354	Cuba	2001
KT726355	Cuba	2001
KT726356	Cuba	2001
KT726357	Cuba	2001
KT726358	Cuba	2001
KT726359	Cuba	2001
KT726360	Cuba	2001
KT726361	Cuba	2002
KU216208	India	2013–11–11
KU216209	India	2013–11–11
KU509278	Barbados	2007
KU509281	India	2009
KU509282	Senegal	2009
KU509283	Sri Lanka	2006
KU509286	India	2011
KX380841	Singapore	2012
KX380842	Singapore	2013
KX855927	India	2014–10–15
KY921907	Singapore	2015–04
LC379193	Gabon	2016–05–15
LC379194	Gabon	2016–05–20
LC379195	Gabon	2016–07–14
LC379196	Gabon	2016–07–22
LC379197	Gabon	2017–04–15
LC410192	Thailand	2016–10
LC410193	Thailand	2016–12
LC410194	Thailand	2016–12
LC410195	Thailand	2017–01
LT898451	Malaysia	2011
LT898452	Malaysia	2011
LT996904	Malaysia	2007
LT996905	Malaysia	2008
LT996906	Malaysia	2008
LT996907	Malaysia	2010
LT996908	Malaysia	2010
LT996909	Malaysia	2010
LT996910	Malaysia	2010
LT996911	Malaysia	2010
LT996912	Malaysia	1987
MF142763	Thailand	2015–09
MF370226	China	2013–08–20
MG721059	India	2016
MG721061	India	2016
MG721064	India	2016
MH048677	Malaysia	2014–12
MH051731	Malaysia	2014–12
MH051733	Malaysia	2014–12
MH544647	Colombia	2015–08–23
MH544649	Colombia	2015–09–15
MH544650	Colombia	2015–09–07
MH544651	Colombia	2016–04–16
MH822957	India	2013
MH888333	Bolivia	2011–01–01
MH891766	India	2017–02–21
MK858149	India	2016–08–26
MK858150	India	2016–08–10
MK858151	India	2016–11–08
MK858152	India	2016–08–24
MK858153	India	2016–10–14
MK858154	India	2017–10–12
MK858155	India	2017–10–09

Accession	Country	Collection date
MK894339	China	2018–04–09
MK894340	China	2018–10–16
MK894341	China	2018–12–30
MN018367	China	2015–09–13
MN018368	China	2013–07–25
MN018371	China	2015–08–08
MN018372	China	2016–03–18
MN018375	China	2015–10–23
MN018376	China	2015–07–06
MN018378	China	2015–09–30
MN018381	China	2016–05–06
MN018385	China	2016–09–20
MN018386	China	2013–07–28
MN227697	China	2019–05–19
MN227698	China	2019–07–02
MN227699	China	2019–06–30
MN227700	China	2019–07–08
MN227701	China	2019–07–08
MN227702	China	2019–06–01
MN227703	China	2019–07–18
MN253124	India	2017–08–10
MN253125	India	2017–10–27
MN253126	India	2016
MN253127	India	2018–09–06
MN253128	India	2018–09–18
MN253129	India	2018–09–20
MN253130	India	2018–12–09
MN253131	India	2018–12–09
MN253132	India	2018–09–18
MN253133	India	2018–10–13
MN448966	Thailand	2012–09–25
MN448967	Thailand	2012–09–25
MN448986	Thailand	2011–06–14
MN448991	Thailand	2012–05–11
MN448992	Thailand	2012–10–01
MN453624	Singapore	2016–01–20
MN922036	China	2019–07–17
MN964273	China	2019–11–09
MN964274	China	2019–11–21
MT261972	Burkina Faso	2017–10–16
MT261973	Burkina Faso	2017–11–09
MT261974	Burkina Faso	2017–11–13
MT261975	Burkina Faso	2017–11–17
MT261976	Burkina Faso	2017–10–04
MT261977	Burkina Faso	2017–10–12
MT261978	Burkina Faso	2017–10–27
MT261979	Burkina Faso	2017–11–02
MW192820	India	2016–08
MW192821	India	2016–08
MW192822	India	2016–08
MW192823	India	2016–07
MW192824	India	2017–08
MW288025	Senegal	2018–10
MW288026	Senegal	2018–10
MW288027	Senegal	2018–10
MW288028	Senegal	2018–10
MW288031	Senegal	2018–11
MW288033	Senegal	2018–11
MW288035	Senegal	2018–11
MW288037	Senegal	2018–11
MW288038	Senegal	2018–11
MW288039	Senegal	2018–11
MW288040	Senegal	2018–11
MW720883	China	2019–09
MW720884	China	2019–09
MW720885	China	2019–09
MW720886	China	2019–09
MW720887	China	2019–09
MW720888	China	2019–09

Accession	Country	Collection date
MW788883	Myanmar	2017–06
MW788884	Myanmar	2017–07
MW788886	Myanmar	2017–07
MW788887	Myanmar	2017–07
MW788888	Myanmar	2017–07
MW788889	Myanmar	2018–07
MW788891	Myanmar	2019–12
MW788893	Myanmar	2019–07
MW788896	Myanmar	2019–05
MW788904	Myanmar	2018–07
MW788906	Myanmar	2018–06
MW788907	Myanmar	2018–10
MW788910	Myanmar	2018–08
MW788911	Myanmar	2018–07
MW945428	Puerto Rico	2006
MW946612	Thailand	2010
MW946615	Thailand	2014
MW946633	Thailand	2013
MW946659	Thailand	2013
MW946662	Thailand	2013
MW946679	Thailand	2014
MW946690	Thailand	2014
MW946693	Thailand	2012
MW946694	Thailand	2014
MW946704	Thailand	2013
MW946711	Thailand	2012
MW946721	Thailand	2011
MW946741	Thailand	2014
MW946763	Thailand	2013
MW946767	Thailand	2006
MW946775	Thailand	2014
MW946778	Thailand	2013
MW946783	Thailand	2012
MW946798	Thailand	2013
MW946799	Thailand	2012
MW946800	Thailand	2012
MW946801	Thailand	2014
MW946802	Thailand	2014
MW946808	Thailand	2010
MW946810	Thailand	2012
MW946815	Thailand	2006
MW946839	Thailand	2011
MW946841	Thailand	2012
MW946843	Thailand	2014
MW946870	Thailand	2014
MW946872	Thailand	2014
MW946881	Thailand	2014
MW946891	Thailand	2011
MW946918	Nicaragua	2009
MW946957	Thailand	2013
MW946958	Thailand	2002
MW946973	Thailand	2013
MW946974	Thailand	2011
MW946979	Thailand	2014
MZ008468	Nicaragua	2014
MZ008469	Nicaragua	2013
MZ008470	Nicaragua	2013
MZ008471	Nicaragua	2013
MZ008472	Nicaragua	2013
MZ008473	Nicaragua	2013
MZ008474	Nicaragua	2013
MZ008475	Nicaragua	2013
MZ008476	Nicaragua	2014
MZ008477	Nicaragua	2013
MZ008478	Nicaragua	2013
MZ312921	India	2018–07–09
MZ544585	Kenya	2019–03
MZ544586	Kenya	2019–03
MZ544587	Kenya	2019–03

Accession	Country	Collection date
MZ544588	Kenya	2019–03
MZ857204	Kenya	2011
MZ857217	Saudi Arabia	2016
OK605762	Paraguay	2007
OK605765	Ecuador	2001
OK605766	Somalia	1993
OM368353	China	2019–05–17
OM417340	Mexico	2021–09–21
OM417341	Mexico	2021–09–19
OM638675	India	2021–11–29
OM865777	Bhutan	2019–07–29
OM865778	Bhutan	2019–07–29
OM865779	Bhutan	2019–07–29
OM865780	Bhutan	2019–07–30
OM865781	Bhutan	2019–07–31
OM865782	Bhutan	2019–08–03
OM865783	Bhutan	2019–08–02
OM865784	Bhutan	2019–08–08
OM865785	Bhutan	2019–08–08
OM865786	Bhutan	2019–08–03
OM865787	Bhutan	2019–08–06
OM865788	Bhutan	2019–08–09
OM865789	Bhutan	2019–08–07
OM865790	Bhutan	2019–08–09
OM865791	Bhutan	2019–08–10
OM865792	Bhutan	2019–08
OM865793	Bhutan	2019–07–19
OM865794	Bhutan	2019–07–27
OM865795	Bhutan	2019–08–24
OM865796	Bhutan	2019–08–20
OM865797	Bhutan	2019–08–20
OM865798	Bhutan	2019–08–22
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OM865801	Bhutan	2019–08–19
OM865802	Bhutan	2019–07–17
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ON007080	Thailand	2011–08–04
ON055565	Thailand	2010–02–16
ON055567	Thailand	2011–08–25
ON109599	India	2021
ON123655	India	2020
ON123658	India	2019
ON123659	India	2018
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ON123665	India	2018
ON123669	India	2018
ON123670	India	2018
ON799401	India	2018
ON890788	Ethiopia	2019–11–09
ON890789	Maldives	2019–05–19

Accession	Country	Collection date
ON890819	China	2019–07–08
ON891145	China	2019–06–01
ON900159	China	2019–06–01
ON907582	China	2019–07–08
ON908232	Ethiopia	2019–11–09
ON908233	Maldives	2019–05–19
ON908234	China	2019–07–08
ON908245	China	2019–06–01
OP410993	Singapore	2008–09
OP410994	Singapore	2013–01
OP410997	Singapore	2019–01
OP410998	Singapore	2018–02
OP895705	India	2018
OP895928	Maldives	2019–03
OP895929	Maldives	2019–12
OP921002	India	2022–07–27
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OQ445899	Cuba	2022–07–28
OQ445901	Cuba	2022–07–25
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OQ445903	Cuba	2022–07–19
OQ445904	Cuba	2022–07–30
OQ445905	Cuba	2022–08–02
OQ445906	Cuba	2022–08–03
OQ445907	Cuba	2022–08–03
OQ445908	Cuba	2022–07–27
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OQ445912	Cuba	2022–08–09
OQ445913	Cuba	2022–08–09
OQ445914	Cuba	2022–08–04
OQ445915	Cuba	2022–08–10
OQ445916	Cuba	2022–08–10
OQ445917	Cuba	2022–08–08
OQ445918	USA	2022–08–09
OQ445919	USA	2022–08–15
OQ445920	USA	2022–08–18
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OQ445927	USA	2022–09–25
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OQ445944	Cuba	2022–07–22
OQ445945	Cuba	2022–07–17
OQ445946	USA	2022–08–09
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OQ445954	Cuba	2022–07–08
OQ445955	Cuba	2022–07–09
OQ445956	Cuba	2022–07–06
OQ445957	Cuba	2022–06–25
OQ445958	Cuba	2022–06–28
OQ445959	Cuba	2022–06–29
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OQ445961	Cuba	2022–05–22
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OQ836208	Puerto Rico	2022–12–27
OQ836209	Puerto Rico	2023–01–24
OQ836210	Puerto Rico	2022–07–30
OQ836211	Puerto Rico	2022–07–27
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OR150747	USA	23.12.22
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OR150750	USA	10.01.23
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OR150755	USA	08.01.23
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OR162313	USA	27.11.22
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OR162316	USA	02.12.22
OR162317	USA	04.12.22
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OR162319	USA	18.12.22
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OQ603309	Colombia	15.09.15
OQ603310	Colombia	2015
OQ603311	Colombia	2015
OQ603312	Colombia	21.01.16
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OR029719	China	27.07.19
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OQ919692	Cuba	04.12.22

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OQ857507	USA	07.11.22
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OQ821559	Cuba	29.09.22
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OQ821563	Cuba	05.10.22
OQ821564	Cuba	07.10.22
OQ821565	Cuba	14.10.22
OQ821566	Cuba	15.10.22

Accession	Country	Collection date
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OQ821569	Cuba	21.10.22
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OQ821571	Cuba	03.11.22
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OQ821573	Cuba	04.11.22
OQ821574	Cuba	07.11.22
OQ821575	Cuba	10.11.22
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OQ821578	Cuba	18.11.22
OQ821579	Cuba	20.11.22
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OQ821599	Cuba	22.12.22
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OQ821602	Jamaica	27.07.16
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OQ821623	USA	23.12.22
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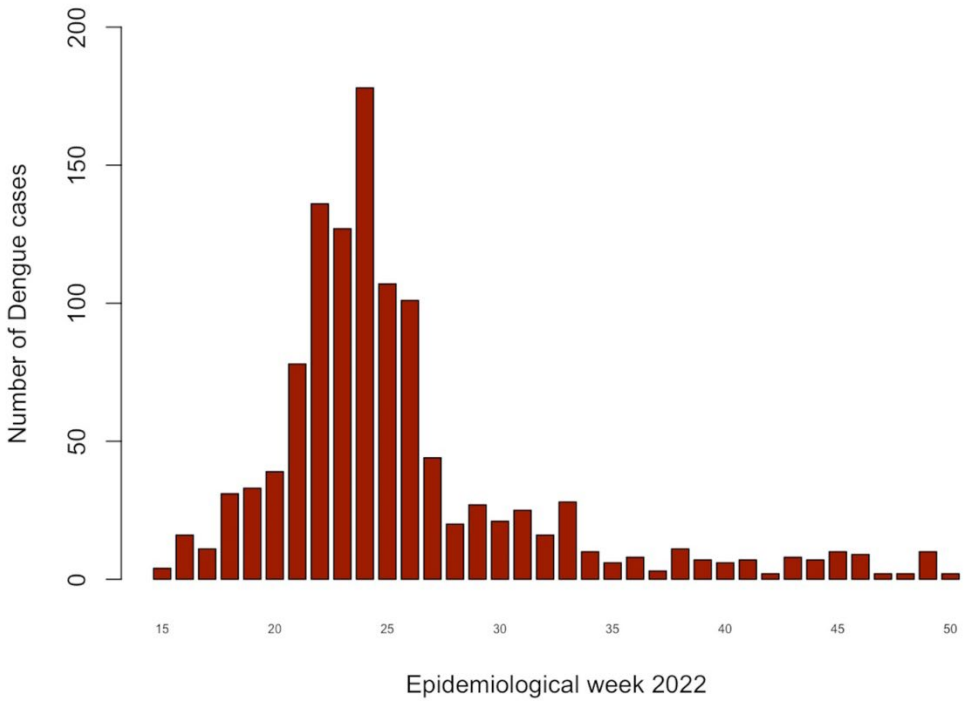
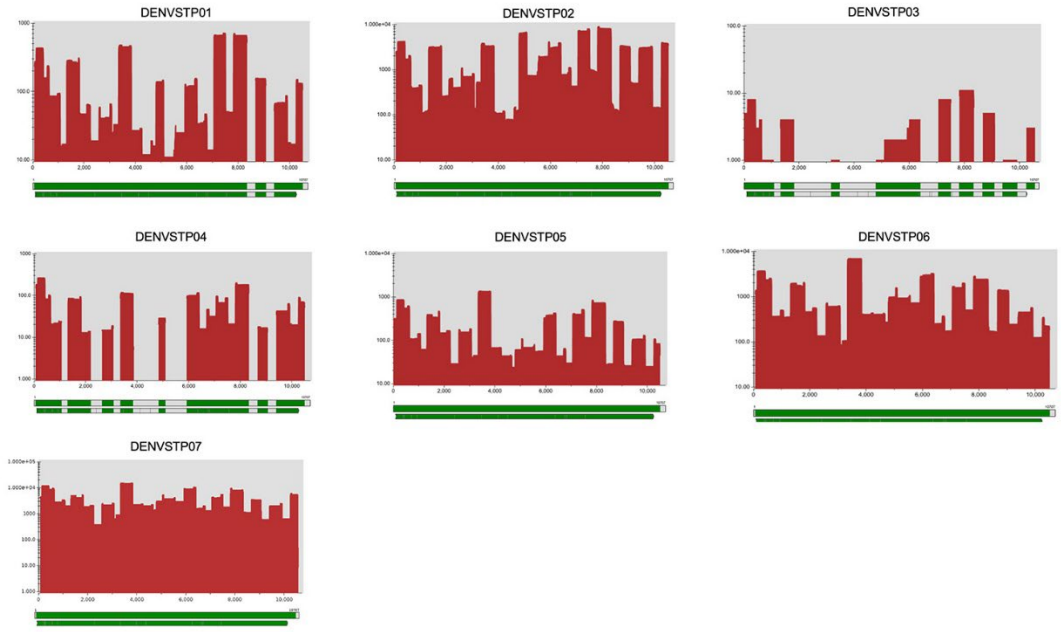
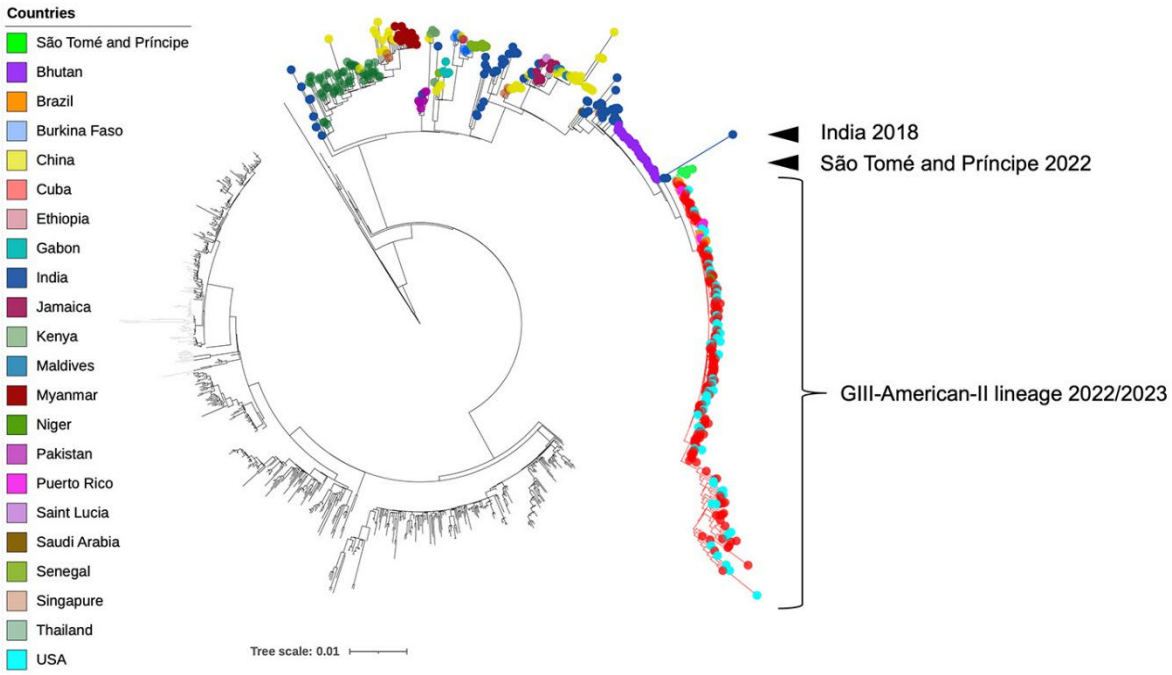


Appendix Figure 1. Epidemiological curve of the Dengue virus outbreak in the Democratic Republic of São Tomé and Príncipe, 2022. Notified Dengue disease cases were confirmed by a positive rapid test.



Appendix Figure 2. Genome coverage plots for seven sequenced DENV-3 GIII isolates from the Dengue virus outbreak in São Tomé and Príncipe, 2022. Assembled to reference NC_001475.



Appendix Figure 3. Unrooted phylogenomic tree including the four DENV-3 genomes from the Dengue outbreak in São Tomé and Príncipe in 2022 and 1,168 DENV-3 G III genomes sampled worldwide. The four STP sequences are clustered and branched between virus genomes isolated in India 2018 (dark blue) and the branch of the recent GIII-American-II lineage comprising isolates from Cuba (red), Brazil (orange), USA (light blue), and Puerto Rico (pink). Scale bar indicates nucleotide substitutions per site.