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GENOME SEQUENCES

Coding-Complete Genome Sequences of 23 SARS-CoV-2 Samples from the Philippines

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ABSTRACT Here, we report the coding-complete genome sequences of 23 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) samples from the Philippines. Sequences were obtained from nasopharyngeal and oropharyngeal swabs from coronavirus disease 2019 (COVID-19)-positive patients. Mutation analysis showed the presence of the D614G mutation in the spike protein in 22 of 23 genomes.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belonging to the family *Coronaviridae* and the genus *Betacoronavirus*, is the causative agent of coronavirus disease 2019 (COVID-19) (1). In the Philippines, the first confirmed SARS-CoV-2 case was reported on 30 January 2020 (2), and as of 31 August 2020, more than 200,000 cases had been reported (3). Here, we announce the coding-complete genome sequences of 23 SARS-CoV-2 samples collected between 3 April and 18 July 2020 from COVID-19 reverse transcriptase PCR (RT-PCR)-positive Filipino patients (4). Viral RNA was extracted from nasopharyngeal/oropharyngeal swabs using a QIAamp viral RNA minikit (Qiagen) and used as the template for amplicon sequencing using ARTIC SARS-CoV-2 V3 primers (4). DNA libraries were constructed and multiplexed using an Illumina DNA prep kit and then pooled before sequencing. Sequencing was performed on a MiSeq instrument using the MiSeq reagent kit v.2 (Illumina). Reference mapping was performed with the Burrows-Wheeler Aligner MEM algorithm (BWA-MEM) using the Wuhan-Hu-1 genome sequence (GenBank accession no. NC_045512.2) as the reference sequence (5). Primer region trimming and call variants (Q, ≥25) were performed using iVar v.1.2.2 (6) and SAMtools (7), respectively. Consensus sequences were generated using iVar v.1.2.2 (6) (Q, ≥25; depth of coverage, ≥10). Gaps, deletions, and ambiguous bases were identified and confirmed by genome-guided assembly with the reference sequence using Trinity v.2.8.5 (8) and Sanger sequencing. The lineage and clade were determined using Pangolin v.2.0.4 (9), GISAID clade nomenclature (10), and phylogenetic analysis (11–13). We used MEGA v.7.0 (14) to examine nucleotide and amino acid substitutions. All tools were run with default parameters. The raw reads yielded a total of 8.4 gigabases (94% of the clusters with a Phred quality score [Q] of ≥30). The reads obtained were 35 to 251 nucleotides long, and 0.87 to 1.48 million reads were obtained per library. Consensus sequence lengths were similar to those of the Wuhan-Hu-1 strain, with the mean coverage ranging from 2,361× to 7,177×.

Table 1 shows the strain details and mutations. A total of 20/23 genomes collected between 25 June and 18 July 2020 were classified under clade GR/lineage B.1.1, a major lineage frequently found in Europe and exported to the rest of the world (9, 10). Two genomes (2/23) from 6 and 7 July 2020 were classified under clade GR/lineage

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TABLE 1 Genome features of 23 samples of SARS-CoV-2 from the Philippines

Sequence name	GenBank and SRA accession no.	Collection date (day-mo-yr)	C_T value[a]	Location	Initial clinical presentation/ classification/ outcome	Genome size (bp)	GC content (%)	Mean depth of coverage (×)	Coverage (%)	Clade	Lineage	Nucleotide substitution	Gene region: amino acid substitution
SARS-CoV-2/ Philippines/ AFRIMS-COVID-GS	MT919768, SRS7273361	3-Apr-20	25.27	Makati City, National Capital Region	Moderate/admitted/ recovered	29,903	37.9	4,508	99.8	O	B.6	C6312A	ORF1a: T2016K
												G11083T	ORF1a: L3606F
												C13730T	ORF1b: A88V
												C23929T	S: synonymous mutation
												C26224T	Nontranslation region
												C28311T	N: P13L
SARS-CoV-2/ Philippines/ AFRIMS-COVID-3304	MT919769, SRS7273362	25-Jun-20	18.80	Quezon City, National Capital Region	Unknown/admitted/ recovered	29,903	38.0	4,958	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												G11083T	ORF1a: L3606F
												C13051T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												C16726T	ORF1b: H1087Y
												C21597T	S: S12F
												A23403G	S: D614G
												A27224C	ORF6: Q8P
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												C29144T	N: synonymous mutation
SARS-CoV-2/ Philippines/ AFRIMS-COVID-3353	MT919770, SRS7273372	26-Jun-20	18.93	Quezon City, National Capital Region	Mild/admitted/ recovered	29,903	38.0	4,134	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												A3938C	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												A23403G	S: D614G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												G28908T	N: G212V
SARS-CoV-2/ Philippines/ AFRIMS-COVID-3477	MT919771, SRS7273376	29-Jun-20	24.74	Taguig City, National Capital Region	Moderate/admitted/ recovered	29,903	38.0	3,856	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												C6726T	ORF1a: T2154I
												C14408T	ORF1b: P314L
												G14414T	ORF1b: S316I
												C21597T	S: S12F
												A23403G	S: D614G
												C26607T	M: L29F
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												C29144T	N: synonymous mutation
												G29543T	Nontranslation region
												G29654A	ORF10: V33I
SARS-CoV-2/ Philippines/ AFRIMS-COVID-3493	MT919772, SRS7273377	29-Jun-20	20.97	Quezon City, National Capital Region	Mild/admitted/ recovered	29,903	38.0	4,448	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												A3938C	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												A23403G	S: D614G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
SARS-CoV-2/ Philippines/ AFRIMS-COVID-3563	MT919773, SRS7273378	29-Jun-20	23.29	Pasay City, National Capital Region	Unknown/ quarantine facility/ recovered	29,903	38.0	5,154	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												G11083T	ORF1a: L3606F
												C13051T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												C16726T	ORF1b: H1087Y
												C21597T	S: S12F
												A23403G	S: D614G
												A27224C	ORF6: Q8P
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												C29144T	N: synonymous mutation

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TABLE 1 (Continued)

Sequence name	GenBank and SRA accession no.	Collection date (day-mo-yr)	C_T value[a]	Location	Initial clinical presentation/ classification/ outcome	Genome size (bp)	GC content (%)	Mean depth of coverage (×)	Coverage (%)	Clade	Lineage	Nucleotide substitution	Gene region: amino acid substitution
SARS-CoV-2/ Philippines/ AFRIMS-COVID-3593	MT919774, SRS7273379	29-Jun-20	22.11	Quezon City, National Capital Region	Mild/admitted/ recovered	29,903	38.0	5,987	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												C7165T	ORF1a: synonymous mutation
												G11083T	ORF1a: L3606F
												C13051T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												C21597T	S: S12F
												A23403G	S: D614G
												C23934T	S: T791I
												A27224C	ORF6: Q8P
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												C29144T	N: synonymous mutation
SARS-CoV-2/ Philippines/ AFRIMS-COVID-4149	MT919775, SRS7273380	6-Jul-20	27.40	Quezon City, National Capital Region	Moderate/unknown/ unknown	29,903	37.9	5,774	99.8	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												C7086T	ORF1a: T2274I
												C9474T	ORF1a: A3070V
												G11083T	ORF1a: L3606F
												C13051T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												C21597T	S: S12F
												A23403G	S: D614G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												C29144T	N: synonymous mutation
SARS-CoV-2/ Philippines/ AFRIMS-COVID-4281	MT919776, SRS7273381	6-Jul-20	18.01	Pasay City, National Capital Region	Mild/quarantine facility/ recovered	29,903	38.0	4,402	99.7	GR	B.1.1.28	G942A	ORF1a: R226K
												C3037T	ORF1a: synonymous mutation
												G3641A	ORF1a: E1126K
												C12053T	ORF1a: L3930F
												C14408T	ORF1b: P314L
												C20133T	ORF1b: synonymous mutation
												A23403G	S: D614G
												G25088T	S: V1176F
												G27816A	ORF7b: V21I
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
SARS-CoV-2/ Philippines/ AFRIMS-COVID-4282	MT919777, SRS7273382	6-Jul-20	17.81	Pasay City, National Capital Region	Asymptomatic/ quarantine facility/ recovered	29,903	38.0	5,454	99.7	GR	B.1.1	C2973T	ORF1a: A903V
												C3037T	ORF1a: synonymous mutation
												C3411T	ORF1a: A1049V
												C10138T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												C20312T	ORF1b: A2282V
												C21597T	S: S12F
												A23403G	S: D614G
												C28308G	N: A12G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												21-nucleotide deletion (28890-28910)	N: S206Y and 7-amino acid deletion (207-PARMAGN-213)
												C29144T	N: synonymous mutation
SARS-CoV-2/ Philippines/ AFRIMS-COVID-4402	MT919778, SRS7273360	7-Jul-20	24.84	Pasay City, National Capital Region	Asymptomatic/ admitted/ recovered	29,903	38.0	6,836	99.7	GR	B.1.1.28	G942A	ORF1a: R226K
												C3037T	ORF1a: synonymous mutation
												G3641A	ORF1a: E1126K
												C12053T	ORF1a: L3930F
												C14408T	ORF1b: P314L
												C20133T	ORF1b: synonymous mutation

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TABLE 1 (Continued)

Sequence name	GenBank and SRA accession no.	Collection date (day-mo-yr)	C_T value[a]	Location	Initial clinical presentation/ classification/ outcome	Genome size (bp)	GC content (%)	Mean depth of coverage (×)	Coverage (%)	Clade	Lineage	Nucleotide substitution	Gene region: amino acid substitution
												A23403G	S: D614G
												G25088T	S: V1176F
												G27816A	ORF7b: V21I
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
SARS-CoV-2/ Philippines/ AFRIMS-COVID-4650	MT919779, SRS7273363	10-Jul-20	26.60	Cavite City, Region 4A (Cavite)	Severe/admitted/ recovered	29,903	38.0	4,987	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												A3938C	ORF1a: synonymous mutation
												C5575T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												A19767G	ORF1b: synonymous mutation
												A23403G	S: D614G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												G28908T	N: G212V
SARS-CoV-2/ Philippines/ AFRIMS-COVID-4792	MT919780, SRS7273364	10-Jul-20	17.74	Calamba City, Region 4A (Laguna)	Asymptomatic/ quarantine facility/ recovered	29,903	37.9	6351	99.8	GR	B.1.1	C2973T	ORF1a: A903V
												C3037T	ORF1a: synonymous mutation
												C3411T	ORF1a: A1049V
												G11083T	ORF1a: L3606F
												C14408T	ORF1b: P314L
												C20312T	ORF1b: A2282V
												C21597T	S: S12F
												A23403G	S: D614G
												C28308G	N: A12G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												21-nucleotide deletion (28890-28910)	N: S206Y and 7-amino acid deletion (207-PARMAGN-213)
												C29144T	N: synonymous mutation
SARS-CoV-2/ Philippines/ AFRIMS-COVID-4837	MT919781, SRS7273366	13-Jul-20	28.30	Taguig City, National Capital Region	Asymptomatic/self-quarantine/ recovered	29,903	38.0	2,361	99.7	GR	B.1.1	C539T	ORF1a: L92F
												C3037T	ORF1a: synonymous mutation
												C8818T	ORF1a: synonymous mutation
												A10912G	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												G17347T	ORF1b: V1294F
												T21198C	ORF1b: synonymous mutation
												C22120T	S: synonymous mutation
												A23403G	S: D614G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												G29540T	Nontranslation region
SARS-CoV-2/ Philippines/ AFRIMS-COVID-5127	MT919782, SRS7273365	14-Jul-20	30.22	Taguig City, National Capital Region	Severe/unknown/ unknown	29,903	38.0	6,780	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												C4039T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												C16559A	ORF1b: A1031E
												G16808A	ORF1b: S1114N
												C19983T	ORF1b: synonymous mutation
												C21575T	S: L5F
												A23403G	S: D614G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												G28899T	N: R209I

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TABLE 1 (Continued)

Sequence name	GenBank and SRA accession no.	Collection date (day-mo-yr)	C_T value[a]	Location	Initial clinical presentation/ classification/ outcome	Genome size (bp)	GC content (%)	Mean depth of coverage (×)	Coverage (%)	Clade	Lineage	Nucleotide substitution	Gene region: amino acid substitution
SARS-CoV-2/ Philippines/ AFRIMS-COVID-5524	MT919783, SRS7273367	15-Jul-20	24.04	Caloocan City, National Capital Region	Unknown/self-quarantine/ recovered	29,903	38.0	7,110	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												G5924A	ORF1a: V1887I
												G6943T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												C18877T	ORF1b: synonymous mutation
												T21775G	S: synonymous mutation
												A23403G	S: D614G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
SARS-CoV-2/ Philippines/ AFRIMS-COVID-5658	MT919784, SRS7273368	16-Jul-20	20.85	Rodriguez, Region 4A (Rizal)	Moderate/admitted/ recovered	29,903	37.9	5,351	99.8	GR	B.1.1	C2973T	ORF1a: A903V
												C3037T	ORF1a: synonymous mutation
												C11572T	OFR1a: synonymous mutation
												C14408T	ORF1b: P314L
												C18828T	ORF1b: synonymous mutation
												C20312T	ORF1b: A2282V
												C21597T	S: S12F
												A23403G	S: D614G
												C26464T	E: synonymous mutation
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												C29144T	N: synonymous mutation
SARS-CoV-2/ Philippines/ AFRIMS-COVID-5698	MT919785, SRS7273369	16-Jul-20	19.37	San Juan City, National Capital Region	Unknown/quarantine facility/recovered	29,903	38.0	6,330	99.7	GR	B.1.1	C593T	ORF1a: H110Y
												C3037T	ORF1a: synonymous mutation
												G5924A	ORF1a: V1887I
												C6525T	ORF1a: T2087I
												G6943T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												G17278T	ORF1b: V1271L
												C18395T	ORF1b: A1643V
												T21775G	S: synonymous mutation
												A23403G	S: D614G
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
SARS-CoV-2/ Philippines/ AFRIMS-COVID-5918	MT919786, SRS7273370	17-Jul-20	27.90	Taguig City, National Capital Region	Severe/admitted/died	29,903	37.9	6,038	99.8	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												C6726T	ORF1a: T2154I
												G11083T	ORF1a: L3606F
												C13051T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												C21597T	S: S12F
												A23403G	S: D614G
												A27224C	ORF6: Q8P
												G27632C	ORF7a: R80T
												G28378A	N: synonymous mutation
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												C29144T	N: synonymous mutation
SARS-CoV-2/ Philippines/ AFRIMS-COVID-5976	MT919787, SRS7273371	18-Jul-20	20.32	Quezon City, National Capital Region	Asymptomatic/ admitted/recovered	29,903	38.0	6,527.5	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												G11083T	ORF1a: L3606F
												C13051T	ORF1a: synonymous mutation
												C14408T	ORF1b: P314L
												C21597T	S: S12F
												A23403G	S: D614G
												A27224C	ORF6: Q8P

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TABLE 1 (Continued)

Sequence name	GenBank and SRA accession no.	Collection date (day-mo-yr)	C_T value[a]	Location	Initial clinical presentation/ classification/ outcome	Genome size (bp)	GC content (%)	Mean depth of coverage (×)	Coverage (%)	Clade	Lineage	Nucleotide substitution	Gene region: amino acid substitution
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
												C29144T	N: synonymous mutation
SARS-CoV-2/ Philippines/ AFRIMS-COVID-5983	MT919788, SRS7273373	18-Jul-20	22.27	Pasay City, National Capital Region	Mild/quarantine facility/recovered	29,903	38.0	6,974.4	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												A3938C	ORF1a: synonymous mutation
												G7042T	ORF1a: M2259I
												C7735T	ORF1a: synonymous mutation
												A9782G	ORF1a: S3173G
												C14408T	ORF1b: P314L
												A23403G	S: D614G
												C28093T	ORF8: S67F
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
SARS-CoV-2/ Philippines/ AFRIMS-COVID-5984	MT919789, SRS7273374	18-Jul-20	22.96	Quezon City, National Capital Region	Unknown/quarantine facility/recovered	29,903	38.0	7,177	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												A3938C	ORF1a: synonymous mutation
												G7042T	ORF1a: M2259I
												C7735T	ORF1a: synonymous mutation
												A9782G	ORF1a: S3173G
												C14408T	ORF1b: P314L
												A23403G	S: D614G
												C28093T	ORF8: S67F
												G28881A, G28882A	N: R203K
												G28883C	N: G204R
SARS-CoV-2/ Philippines/ AFRIMS-COVID-6009	MT919790, SRS7273375	18-Jul-20	26.55	San Jose del Monte City, Region 3 (Bulacan)	Mild/self-quarantine/ recovered	29,903	38.0	6,970	99.7	GR	B.1.1	C3037T	ORF1a: synonymous mutation
												A3938C	ORF1a: synonymous mutation
												G7042T	ORF1a: M2259I
												C7735T	ORF1a: synonymous mutation
												A9782G	ORF1a: S3173G
												C14408T	ORF1b: P314L
												A23403G	S: D614G
												C26305T	E: L21F
												C28093T	ORF8: S67F
												G28881A, G28882A	N: R203K
												G28883C	N: G204R

[a] C_T, threshold cycle.

B.1.1.28, found in Brazil (89%), the United Kingdom (8%), and China (2%) (https://cov-lineages.org/lineages/lineage_B.1.2.html). A single genome (1/23) was classified under clade O/lineage B.6, a global lineage mostly found in Singapore and India (https://cov-lineages.org). Four amino acid substitutions (ORF1b-P314L, S-D614G, N-R203K, and N-G204R) unique to clade GR (both lineages B.1.1 and B.1.1.28) were seen in all our sequences in this clade. Two sequences of lineage B.1.1 had a 21-nucleotide deletion in the N gene region. Five substitutions (ORF1a-R226K, ORF1a-E1126K, ORF1a-L3930F, S-V1176F, and ORF7b-V21I) were observed uniquely in 2 sequences of lineage B.1.1.28.

None of the Philippine SARS-CoV-2 sequences in GISAID (accessed 22 August 2020) with a collection date before 25 June 2020 (15, 16) contained the D614G mutation. From the same set of data, lineage B.1.1 containing D614G was found in a sample collected on 25 June 2020. This observation coincides with our findings that the D614G mutation was observed in samples collected in June 2020 (16). Our findings show the presence of lineages B.6, B.1.1, and B1.1.28, with the latter being first reported in the Philippines in this study.

The D614G mutation is currently the most prevalent variant worldwide and is associated with higher viral RNA levels and titers of pseudoviruses (17). In this study, all patients had no history of travel outside the country and acquired the disease in the National Capital Region, Region 3, or Region 4A (Table 1), providing evidence of local transmission. Multiple lineages and strains could have been introduced by travelers and Filipino repatriates (18). The D614G mutation is replacing and rapidly surpassing in prevalence the original strain circulating prior to June 2020 and may partially explain the rapid rise of cases in the Philippines.

Data availability. The SARS-CoV-2 genomes from the Philippines were deposited in the GenBank database (accession no. MT919768 to MT919790). The raw reads have been deposited in the NCBI Sequence Read Archive (SRA accession no. SRS7273360 to SRS7273382). The BioProject accession no. is PRJNA659293. The BioSample accession no. are SAMN15903138 to SAMN15903160.

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We declare no competing interests.

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