



First Complete Genome Sequence of Currently Circulating Infectious Bronchitis Virus Strain DMV/1639 of the GI-17 Lineage

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ABSTRACT Avian infectious bronchitis virus is the causative agent of a highly contagious disease that results in severe economic losses to the poultry industry worldwide. Here, we report the first coding-complete genome sequence of strain DMV/1639 of the GI-17 lineage, isolated from broiler chickens in Georgia in 2019.

Infectious bronchitis virus (IBV) is the etiological agent of an acute and highly contagious disease that affects chickens of all ages (1). IBV is a single-stranded positive-sense RNA virus of the family *Coronaviridae*, genus *Gammacoronavirus* (2–4). Genetic diversity in coronaviruses is due to adaptive evolution caused by high mutation rates and genetic recombination (5, 6). Based on the spike 1 (S1) protein variability, six genotypes of IBV comprising 32 distinct viral lineages have been described (7). Numerous IBV strains have been reported in the United States. A new IBV strain, Delmarva/1639 (DMV/1639), of the GI-17 lineage was first detected in broiler flocks on the Delmarva Peninsula in 2011 (8). This IBV strain continues to circulate and typically causes airsacculitis and respiratory illness in affected chickens. To date, only partial viral genomes of the DMV/1639 strain viruses are available in public databases (8, 9). In this study, we report the first isolation of this IBV strain from Georgia and the first coding-complete sequence of a DMV/1639 strain.

The DMV/1639 IBV strain was isolated from a broiler chicken at a commercial farm in Georgia in 2019. Fresh cecal tonsil samples were homogenized and inoculated into specific-pathogen-free embryonating chicken eggs. Total RNA was isolated from allantoic fluid using the Direct-zol RNA miniprep kit (Zymo Research, USA). The Illumina library was prepared using the Kapa stranded RNA sequencing (RNA-seq) library preparation kit (Kapa Biosystems, USA) per the manufacturer's instructions. The distribution size and concentration of the prepared library were checked on a Bioanalyzer 2100, using a high-sensitivity (HS) DNA kit (Agilent Technologies, Germany), and a Qubit fluorometer, using the double-stranded DNA (dsDNA) HS assay kit (Life Technologies, USA), respectively. Next-generation paired-end sequencing (2 × 150 bp) was performed on a MiSeq instrument using the 300-cycle MiSeq reagent kit version 2 (Illumina, USA). Sequence data were assembled using a *de novo* approach and utilizing MIRA version 3.4.1 (10) within a customized workflow on the Galaxy platform (11), as described previously (12, 13). Briefly, the following parameters were specified for the assembly step: assembly method, *de novo*; assembly quality grade, accurate; use read extension, yes; minimum reads per contig, 100; minimum overlap, 16; mark repeats, yes; maximum megahub ratio, 0.2; and spoiler detection, yes. Default settings were used for the rest of the parameters. A total of 2,073,159 raw paired-end reads were generated. The final genome consensus of the isolate, designated GA9977/2019, was 27,672 nucleotides (nt) long and was called from 953,689 IBV reads using BWA-MEM (14). The

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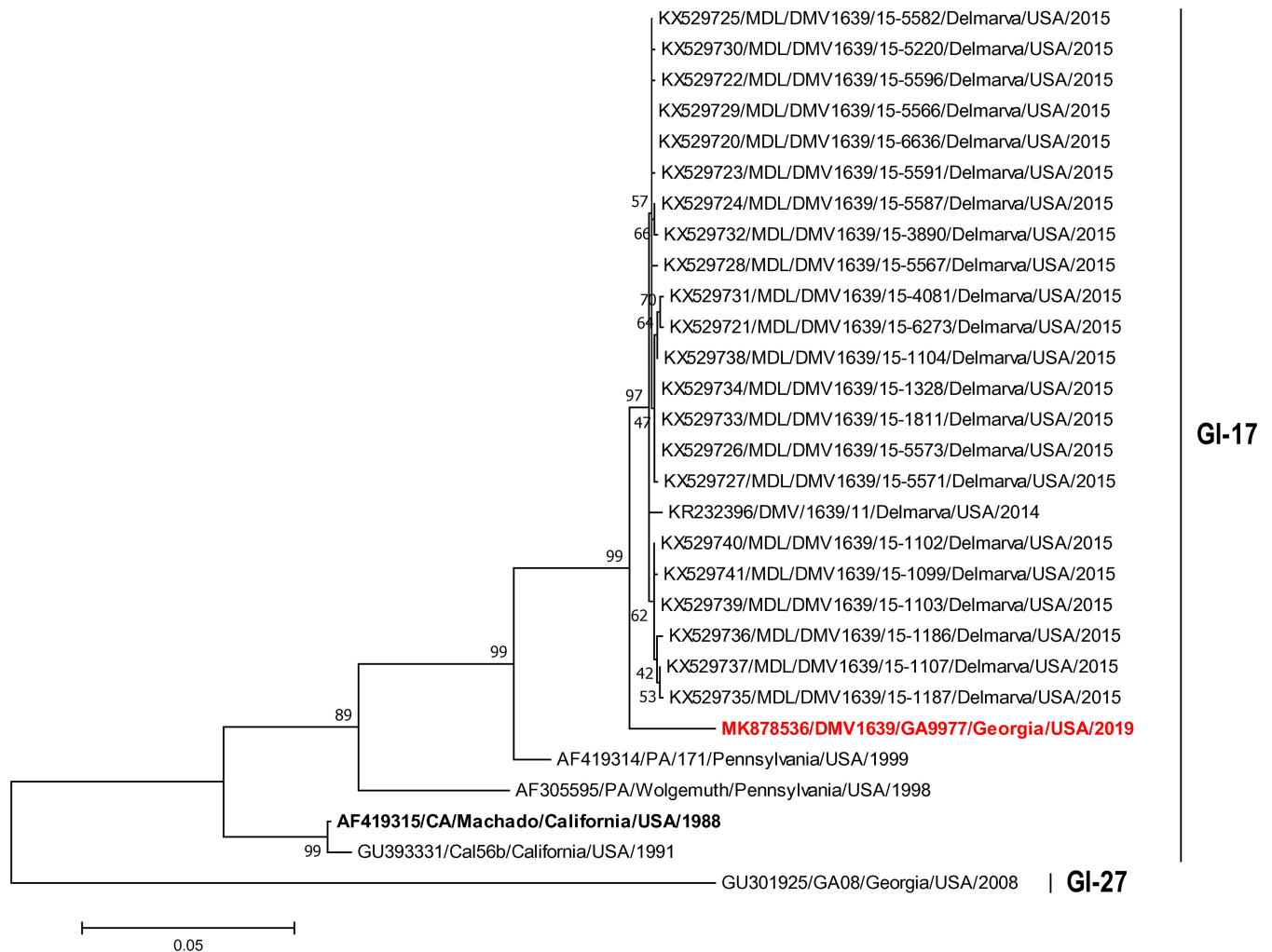


FIG 1 Phylogenetic analysis of IBV isolates of the DMV/1639 strain based on the complete S1 gene sequences constructed with the maximum likelihood method based on the general time-reversible model in MEGA version 7.0. The tree with the highest log likelihood ($-4,651.12$) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The initial tree(s) for the heuristic search was obtained automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter, 0.6631]). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 28.1% sites). The tree is drawn to scale, with branch lengths measured by the number of substitutions per site. The analysis involved 29 nucleotide sequences (sequences from the GI-27 lineage are included as an outgroup). All positions containing gaps and missing data were eliminated. There were a total of 1,608 positions in the final data set.

median read depth of the IBV assembly was 4,326, and the maximum depth was 20,867 reads. Although a very short (2-nt) sequence at the extreme 3' terminus was missing due to complete absence of coverage in the raw data, all coding sequences were obtained, resulting in 99.99% genome coverage. The open reading frames (ORFs) were identified using Geneious 9.1.8 and confirmed by alignment with published IBV genomes. The genome has the typical genetic structure of all IBV strains and contains 13 ORFs (5'-1a-1ab-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-3') of 11,859 nt, 19,893 nt, 3,501 nt, 174 nt, 195 nt, 303 nt, 669 nt, 285 nt, 171 nt, 198 nt, 249 nt, 1,230 nt, and 225 nt in length, respectively. A preliminary BLAST comparison to the currently available full-length IBV genome sequences showed the highest (95.76%) nucleotide identity to the California strain Cal56b/1991 (GenBank accession number [GU393331](#)) belonging to the GI-17 lineage (7, 15–17). Detailed phylogenetic analysis based on the complete coding sequence of the S1 gene (7, 18) confirmed that GA9977/2019 is a member of GI-17 lineage clustering in one group along with the lineage prototype strain CA/Machado/1988 (90.35% nucleotide identity, GenBank accession number [AF419315](#)). The GA9977/

2019 isolate, together with the DMV/1639 isolates from the Delmarva Peninsula, formed a distinct cluster with nucleotide identities ranging from 97.71% to 97.47% (Fig. 1). Currently, there are no IBV reference full-genome sequences available for the DMV/1639 IBV strain isolates, only sequences of the S1 gene (8, 9). This coding-complete genome sequence information would be useful for in-depth understanding of the evolution of IBVs as well as planning vaccination strategies.

Data availability. The coding-complete genome sequence of the GA9977/2019 isolate of the DMV/1639 strain has been deposited in GenBank under the accession number [MK878536](https://www.ncbi.nlm.nih.gov/nuclseq/MK878536). Raw data were deposited in the SRA under accession number [SRR9763527](https://www.ncbi.nlm.nih.gov/sra/SRR9763527), BioSample number [SAMN12347422](https://www.ncbi.nlm.nih.gov/biosample/SAMN12347422), and BioProject number [PRJNA556282](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA556282).

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