

Genomic Characterization of Diverse Gyroviruses Identified in the Feces of Domestic Cats

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Supplementary Table S1. Oligonucleotide sequences and amplification conditions of primers used for the detection of gyroviruses.

| Virus | Nucleotide sequence (3') | Amplicon size (bp) | Amplification program |
|--------------|---------------------------------|---------------------------|--|
| CAV | ATCAACGGTG TTCAGGC | 535 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 57°C for 30 s and 72°C for 45 s, and a final extension at 72 °C for 10 min |
| | CCTTGGAAGCGGATAGTCAT | | |
| HGyV/AGV2 | CGCGTAGAAGATCCTTTGATC | 293 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 30 s, and a final extension at 72 °C for 10 min |
| | CTGCAAGTGCTGAGGATAAGG | | |
| GyV3 | GACACAGACTGCGACGAAGA | 412 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 30 s, and a final extension at 72 °C for 10 min |
| | ATGCTCCTGGCTGTCTAGAT | | |
| GyV4 | GTGGTATCGAAGTGGAAGTACC | 285 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 48°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min |
| | CCCCCTGATACATACTGTACATA | | |
| GyV5 | CCGTGGGGGAGGCTTTACAA | 475 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min |
| | GCACCGCCATCCCAAACAC | | |
| GyV6 | CTTCCGCGACCACAAACCAC | 473 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min |
| | GCGGCCATTATAGCGACCTTAG | | |
| GyV7 | AGGATGGCAACTTTTCAGGC | 224 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min |
| | TGGCTGTGCCGCGCCAGTAGT | | |
| GyV8 | GCGCCGCCATTACAGAGG | 371 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min |
| | CTTTTTGCGGCATATTAGTCCA | | |
| GyV9 | TGAAACACTTATTGGAGA ACTGTACCT | 1841 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 62°C for 30 s and 72°C for 2 min, and a final extension at 72°C for 10 min |
| | TTGTCCTCTCTTTGAGCCTCTGTC | | |
| GyV10 | CCCCGGCTCGTATGTGGTAAGA | 414 | 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min |
| | GGCTGTGCGGGGAAATAGTCAA | | |

Supplementary Table S2. Oligonucleotide sequences of specific primers used for quantitative PCR.

| Virus | Nucleotide sequence (5'-3') | Amplicon size (bp) |
|--------------|------------------------------------|---------------------------|
| CAV | AGCGCAAGGCACAAATAAGTC | 90 |
| | GCCCATGAGTCGCTCTTCA | |
| HGyV/AGV2 | TGGTGCGTGTTGAAAGTC | 96 |
| | CGTTGCAGTGTGCTCGTTGT | |
| GyV3 | TCCACAACACATCCAGG | 140 |
| | AAGAAGAAGTGCCCATCG | |
| GyV6 | TTCTCAGCGTTATCAGCCCTT | 167 |
| | GTGTCATCTGTAATCCATTCG | |

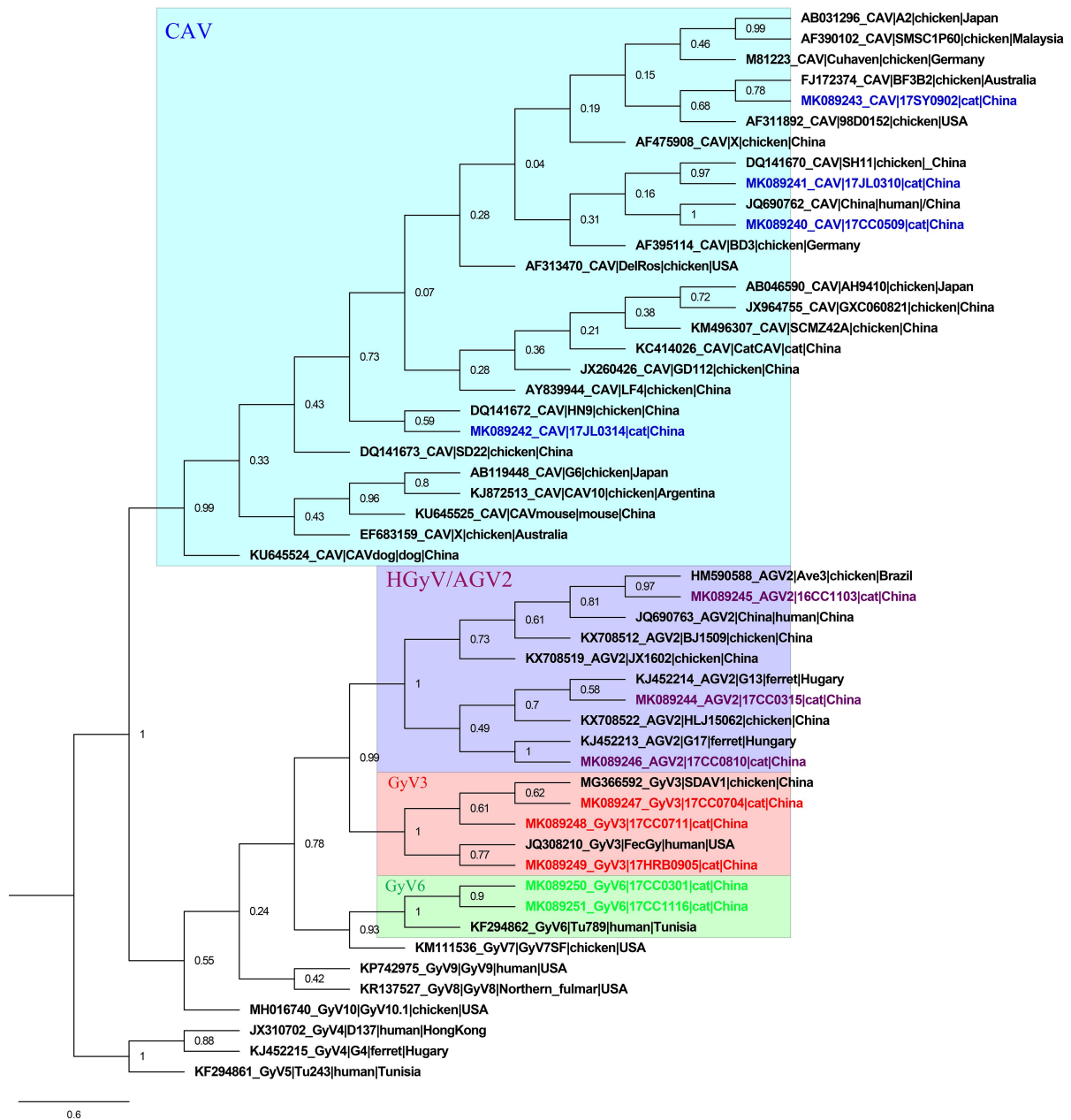
Supplementary Table S3. Primers used for the gyrovirus genome amplification.

| Virus | Nucleotide sequence (5'-3') | Amplicon size (bp) | Amplification program |
|--------------|------------------------------------|---------------------------|---|
| CAV | GCATTCCGAGTGGTTACTATTCC | 843 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 55°C for 50 s and 72°C for 60 s, and a final extension at 72 °C for 10 min |
| | CGTCTTGCCATCTTACAGTCTTAT | | |
| | CGAGTACAGGGTAAGCGAGCTAAA | 989 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 55°C for 50 s and 72°C for 60 s, and a final extension at 72 °C for 10 min |
| | TGCTATTCATGCAGCGGACTT | | |
| | ACGAGCAACAGTACCCTGCTAT | 802 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 55°C for 50 s and 72°C for 60 s, and a final extension at 72 °C for 10 min |
| | CTGTACATGCTCCACTCGTT | | |
| HGyV/AGV2 | TCACAGCCAATCAGAATTGAGCACG | 733 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 50 s and 72°C for 60 s, and a final extension at 72 °C for 10 min |
| | TTCTACGCGCATATCGAAATTTACC | | |
| | TATTCCTCGGAGGGGTAAATTTTCGAT | 981 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 50 s and 72°C for 60 s, and a final extension at 72 °C for 10 min |
| | CCCCTGTCCCCGTGATGGAATGTTT | | |
| | ATTTCTAGCACTCAAAAACCCATT | 802 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 50 s and 72°C for 60 s, and a final extension at 72 °C for 10 min |
| | TCTGGGCGTGCTCAATTCTGATT | | |
| GyV3 | GACACAGACTGCGACGAAGA | 431 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 50 s and 72°C for 30 s, and a final extension at 72 °C for 10 min |
| | ATGCTCCTGGCTGTCTAGAT | | |
| | AAGGTCAAAGATTGGAGGAC | 2203 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 60 s and 72°C for 2 min, and a final extension at 72 °C for 10 min |
| | TAGGTATGAATACGAGCC | | |
| GyV6 | TACTTACGCGCAGCAGATCGT | 1846 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 65°C for 60 s and 72°C for 2 min, and a final extension at 72 °C for 10 min |
| | CGCTCCAAGGGCTGATAACG | | |
| | AATGACAGGCATGATACGC | 594 | 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 55°C for 50 s and 72°C for 30 s, and a final extension at 72 °C for 10 min |
| | CCCATTACCAATCAGCT | | |

Supplementary Table S4. Percentage pairwise sequences identities (nucleotide/amino acid) among cat-origin gyroviruses and representative reference strains. The highest nucleotide and amino acid pairwise identities are shown in red color, the lowest are shown in blue color.

| Isolate | Representative gyrovirus strains | Genome | VP1 | VP2 | VP3 |
|------------------|----------------------------------|---------------|-------------------|-------------------|------------------|
| CAV | | | | | |
| 17CC0509 | JQ690762 (China/Human/China) | 99.3/- | 99.3/99.3 | 99.7/99.1 | 99.7/99.2 |
| | KC414026 (CatCAV/Cat/China) | 98.1/- | 97.9/98.0 | 99.4/99.1 | 99.5/99.2 |
| | DQ141670 (SH11/Chicken/China) | 97.6/- | 97.6/97.3 | 98.9/98.6 | 99.2/98.4 |
| | KU645524 (CAVdog/Dog/China) | 96.3/- | 95.2/ 96.6 | 98.6/98.6 | 98.6/96.8 |
| | KU645525 (CAVmouse/Mouse/China) | 95.8/- | 94.0/97.0 | 98.4/97.2 | 99.2/97.6 |
| | EF683159 (X/Chicken/Australia) | 94.7/- | 93.9/98.0 | 98.4/98.1 | 98.1/95.1 |
| 17JL0310 | JQ690762 (China/Human/China) | 98.0/- | 98.1/97.8 | 99.2/99.5 | 99.5/99.2 |
| | KC414026 (CatCAV/Cat/China) | 98.2/- | 97.9/97.1 | 99.2/98.6 | 99.2/99.2 |
| | DQ141670 (SH11/Chicken/China) | 99.8/- | 99.9/99.8 | 100/100 | 100/100 |
| | KU645524 (CAVdog/Dog/China) | 97.2/- | 96.0/ 96.9 | 99.7/100 | 99.5/98.4 |
| | KU645525 (CAVmouse/Mouse/China) | 96.6/- | 95.0/98.0 | 98.9/ 98.6 | 99.5/99.2 |
| | EF683159 (X/Chicken/Australia) | 95.5/- | 94.8/98.7 | 99.1/99.5 | 98.9/96.7 |
| 17JL0314 | JQ690762 (China/Human/China) | 97.3/- | 97.4/97.8 | 98.9/98.2 | 98.9/98.4 |
| | KC414026 (CatCAV/Cat/China) | 99.1/- | 99.1/98.9 | 99.8/100 | 99.7/100 |
| | DQ141670 (SH11/Chicken/China) | 98.6/- | 98.2/98.0 | 99.4/98.6 | 99.5/99.2 |
| | KU645524 (CAVdog/Dog/China) | 97.1/- | 96.2/97.3 | 99.1/98.6 | 98.9/97.5 |
| | KU645525 (CAVmouse/Mouse/China) | 96.0/- | 94.3/96.9 | 98.6/97.2 | 98.9/98.4 |
| | EF683159 (X/Chicken/Australia) | 95.3/- | 94.8/99.6 | 98.8/98.2 | 98.4/95.9 |
| 17SY0902 | JQ690762 (China/Human/China) | 97.9/- | 98.0/98.0 | 99.1/99.5 | 99.2/98.4 |
| | KC414026 (CatCAV/Cat/China) | 98.5/- | 98.4/98.2 | 99.1/98.6 | 98.9/98.4 |
| | DQ141670 (SH11/Chicken/China) | 99.0/- | 99.0/98.7 | 99.8/100 | 99.7/99.2 |
| | KU645524 (CAVdog/Dog/China) | 97.5/- | 96.4/97.6 | 99.6/99.8 | 99.5/99.2 |
| | KU645525 (CAVmouse/Mouse/China) | 96.8/- | 95.3/97.3 | 98.8/98.6 | 99.2/98.4 |
| | EF683159 (X/Chicken/Australia) | 95.8/- | 95.3/96.8 | 98.9/99.5 | 98.6/95.9 |
| HGyV/AGV2 | | | | | |
| 16CC1103 | JQ690763 (China/Human/China) | 98.0/- | 97.5/97.9 | 99.1/99.1 | 98.7/96.8 |
| | KJ452213 (G17/Ferret/Hungary) | 95.0/- | 93.0/97.4 | 97.1/95.7 | 96.3/92.0 |
| | KJ452214 (G13/Ferret/Hungary) | 95.7/- | 94.5/97.8 | 97.3/97.0 | 96.3/93.6 |
| | HM590588 (Ave3/Chicken/Brazil) | 99.7/- | 99.9/100 | 99.9/100 | 99.7/99.2 |
| 17CC0315 | JQ690763 (China/Human/China) | 95.7/- | 94.6/98.7 | 97.7/96.1 | 97.1/94.4 |
| | KJ452213 (G17/Ferret/Hungary) | 99.2/- | 99.1/99.6 | 99.3/99.1 | 98.9/96.8 |
| | KJ452214 (G13/Ferret/Hungary) | 95.8/- | 94.4/98.3 | 98.6/98.7 | 97.9/94.4 |
| | HM590588 (Ave3/Chicken/Brazil) | 95.6/- | 94.1/97.8 | 97.6/96.1 | 96.5/93.6 |
| 17CC0810 | JQ690763 (China/Human/China) | 96.0/- | 95.2/99.1 | 97.3/96.1 | 96.8/93.6 |
| | KJ452213 (G17/Ferret/Hungary) | 98.9/- | 99.3/99.6 | 99.3/99.1 | 98.7/95.2 |
| | KJ452214 (G13/Ferret/Hungary) | 96.0/- | 93.9/98.3 | 98.6/98.7 | 98.1/94.4 |

| | | | | | |
|-------------|--------------------------------|--------|-----------|-----------|-----------|
| | HM590588 (Ave3/Chicken/Brazil) | 94.8/- | 93.1/97.4 | 97.1/96.1 | 96.3/92.8 |
| GyV3 | | | | | |
| 17CC0704 | JQ308210 (FecGy/Human/USA) | 98.7/- | 98.6/100 | 99.2/98.3 | 99.5/98.4 |
| | MG366592 (SDAV1/Chicken/China) | 99.7/- | 100/100 | 99.9/100 | 100/100 |
| 17CC0711 | JQ308210 (FecGy/Human/USA) | 98.8/- | 98.6/100 | 99.4/99.2 | 99.5/98.4 |
| | MG366592 (SDAV1/Chicken/China) | 99.6/- | 100/100 | 99.6/99.2 | 100/100 |
| 17HRB0905 | JQ308210 (FecGy/Human/USA) | 99.5/- | 100/100 | 100/100 | 99.5/98.4 |
| | MG366592 (SDAV1/Chicken/China) | 98.9/- | 98.6/100 | 99.0/98.3 | 100/100 |
| GyV6 | | | | | |
| 17CC0301 | KF294862 (Tu789/Human/Tunisia) | 98.7/- | 99.1/99.3 | 99.7/99.6 | 100/100 |
| 17CC1116 | KF294862 (Tu789/Human/Tunisia) | 99.1/- | 99.1/99.3 | 99.9/100 | 100/100 |



Supplementary Figure S1. Maximum likelihood phylogenetic tree based on the whole genome nucleotide sequences of the 12 cat-origin gyroviruses and 40 reference gyroviruses. Branch lengths are measured in nucleotide substitutions and numbers show branching percentages in bootstrap replicates. The tree is rooted at the midpoint of the longest branch. Cat-origin CAV, HGyV/AGV2, GyV3 and GyV6 strains identified in the present study are shown in blue, purple, red and green color, respectively.