Novel Orthonairovirus Isolated from Ticks near China-North Korea Border

Fan Li,¹ Jixu Li,¹ Jingdong Song, Qikai Yin, Kai Nie, Songtao Xu, Ying He, Shihong Fu, Guodong Liang, Qiang Wei, Huanyu Wang

We isolated a new orthonairovirus from *Dermacentor silvarum* ticks near the China–North Korea border. Phylogenetic analysis showed 71.9%–73.0% nucleic acid identity to the recently discovered Songling orthonairovirus, which causes febrile illness in humans. We recommend enhanced surveillance for infection by this new virus among humans and livestock.

Viruses of the genus *Orthonairovirus*, family *Nairoviridae*, include the consequential ticktransmitted pathogens Crimean-Congo hemorrhagic fever virus and Nairobi sheep disease virus, as well as other poorly characterized viruses that have been found in ticks and mammals. *Orthonairovirus* virions are spherical in shape (80–120-nm diameter) with 3 single-stranded RNA segments 17.1–22.8 kilobases in length and a membrane envelope (1–5). We performed surveillance in areas endemic for tick-borne encephalitis (6) and identified a novel orthonairovirus from *Dermacentor silvarum* ticks collected in 2021 in Jilin Province, China, near the China-North Korea border.

The Study

On April 17, 2021, we dragged corduroy to collect ticks from a forest region in Antu (118°46′E, 43°15′N), a district of the city of Yanbian in eastern Jilin Province, China, near the border with North Korea. We identified captured ticks according to morphologic keys and stored them at 4°C with wet cotton. We

Author affiliations: Chinese Center for Disease Control and Prevention, Beijing, China (F. Li, J. Song, Q. Yin, K. Nie, S. Xu, Y. He, S. Fu, G. Liang, Q. Wei, H. Wang); Yanbian Korean Autonomous Prefecture Center for Disease Control and Prevention, Jilin, China (J. Li)

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collected 264 ticks of 3 species—29 *Ixodes persulcatus*, 193 *Dermacentor silvarum*, and 12 *Haemaphysalis concinna*—and 30 larvae of unidentified species.

We homogenized ticks using a QIAGEN TissueLyser (QIAGEN, https://www.qiagen.com) and inoculated supernatants onto a monolayer of African green monkey kidney (Vero) E6 cells. After 3 successive passages, we observed cells for cytopathic effects. The inoculate from Dermacentor silvarum ticks, designated as YB_tick_2021_24, caused cytopathic effects in Vero E6 cells 96 h after inoculation (Figure 1, panels A, B). We collected cells showing cytopathic effects, then fixed and embedded them in epoxy resin. We cut ultrathin (80 nm) sections from the resin block, stained them with citrate lead and uranyl acetate, and observed them under a transmission electron microscope. We observed enveloped virus particles ≈100 nm in diameter that shared morphologic features with Bunyavirales viruses (Figure 1, panel C).

We extracted viral RNA from infected culture supernatants using a QIAGEN QIAamp Viral RNA Mini Kit, synthesized cDNA, prepared DNA libraries using an Illumina Nextera XT Kit (Illumina, https://www.illumina.com), and performed 150 bp paired-end sequencing using the Illumina MiniSeq System. We filtered reads on the basis of their length and mean quality values. We prepared contigs by de novo assembly and subjected them to BLASTx alignment (https://blast.ncbi.nlm.nih.gov/Blast. cgi) at E value <10⁻⁴ against the nonredundant protein and viral proteome databases of the National Center for Biotechnology Information. We used Bowtie 2 (https://bowtie-bio.sourceforge.net/bowtie2/index.shtml) to remap the clean reads to the generated virus-related contigs (7). We used rapid

¹These authors contributed equally to this article.

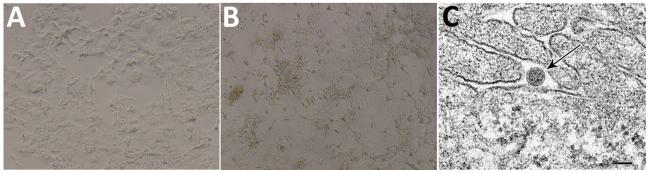
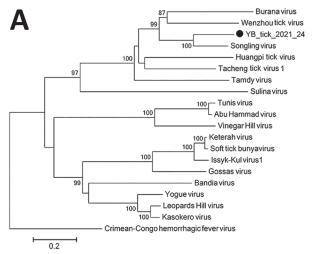


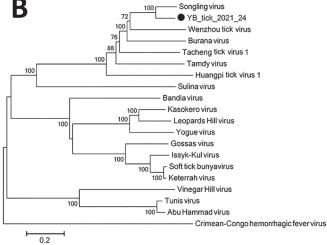
Figure 1. Discovery and characterization of novel orthonairovirus Antu virus isolate YB_tick_2021_24 from *Dermacentor silvarum* ticks in China. A) Vero E6 cells without YB_tick_2021_24 infection. Original magnification ×10. B) YB_tick_2021_24-infected Vero E6 cells showing cytopathic effects visible by light microscopy. Original magnification ×10. C) Ultrathin section electron micrograph of an isolated particle (black arrow) on a cell surface. Scale bar = 100 nM

amplification of cDNA ends (RACE) PCR and Sanger sequencing to confirm the terminal sequences of virus genomes, and deposited the new genome in GenBank (accession nos. OQ207701–3). We identified open read frames (ORFs) using ORF finder (https://www.ncbi.nlm.nih.gov/orffinder) and

calculated sequence similarities using BLAST.

Our procedure generated 40,826,350 reads (6.1 Gbp), which produced 266 virus-related contigs. Three contigs, the 1,516 bp small (S), 3,936 bp medium (M), and 12,133 bp large (L) segments, were annotated to Songling virus (SLV), a previously reported





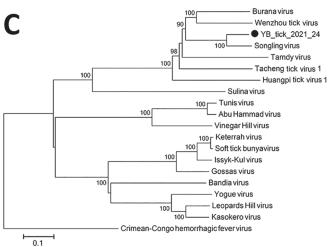


Figure 2. Molecular phylogenetic analysis by neighbor-joining tree based on the amino acid sequences novel orthonairovirus Antu virus isolate YB_tick_2021_24 (black circles) from Dermacentor silvarum ticks in China. A) Small segment; B) medium segment; C) large segment. Numbers associated with branches indicate percentages of 1,000 bootstrap replicates that support the existence of these branches. Branches with <70% bootstrap support have been collapsed. Scale bars represent amino acid substitutions per site.

Table. Homology comparisons of the sequence of novel orthonairovirus Antu viruses from China and other related viruses*

Protein/virus	Antu virus	SLGV	WTV	TTV	BURV	TDYV	HTV	CCHFV
Small								
Antu virus		71.5	52.6	53.7	52.1	48.9	47.4	37.3
SLGV	71.9		55.3	51.2	51.8	49.6	45.9	34.9
WTV	60.3	60.6		50.0	54.1	43.8	46.0	34.0
TTV	59.5	57.9	56.6		46.6	49.1	51.0	33.4
BURV	58.6	59.9	60.5	55.2		44.3	42.4	35.0
TDYV	56.5	58.2	54.0	57.9	53.1		45.0	34.3
HTV	55.5	55.8	55.9	58.7	52.9	55.8		35.2
CCHFV	47.6	46.8	46.8	46.5	47.9	47.7	46.3	
Medium								
Antu virus		79.5	59.9	56.3	58.7	53.8	46.9	25.4
SLGV	72.4		58.2	53.9	57.0	51.2	46.5	24.5
WTV	61.9	61.8		51.8	54.0	50.0	46.5	24.5
TTV	57.2	58	56.4		51.6	51.1	48.1	24.5
BURV	61	61.2	58.9	58		49.4	47.2	24.0
TDYV	56.6	57.5	55.9	55.1	55.4		45.7	24.4
HTV	53.7	53.9	53.4	52.9	54.2	52.1		24.6
CCHFV	41.8	40.7	40.5	40.6	41.3	42.2	40.3	
Large								
Antu virus		84.6	66.5	64.1	66.0	62.2	60.1	39.2
SLGV	73.0		65.7	64.0	65.1	61.4	60.1	38.5
WTV	63.4	63.7		63.5	69.7	61.7	60.0	38.5
TTV	61.9	62.0	62.2		63.2	59.3	60.0	38.7
BURV	63.4	63.3	66.1	61.8		61.5	60.3	38.7
TDYV	60.4	60.5	60.5	59.4	60.8		58.0	39.2
HTV	59.8	60.1	59.5	61.5	60.3	58.2		38.2
CCHFV	48.1	48.3	48.2	48.0	48.8	48.1	48.5	

*Percentage nucleotide sequence identity presented below and amino acid identity above blank cells. BURV, Burana virus; CCHFV, Crimean-Congo hemorrhagic fever virus; HTV, Huangpi tick virus; SGLV, Songling virus; TDYV, Tamdy virus; TTV, Tacheng tick virus; WTV, Wenzhou tick virus.

orthonairovirus (8). Average sequencing coverages remapped to the 3 contigs were 48× (S), 63× (M), and 234× (L). The final genome lengths confirmed by RACE sequencing were 1,848 bp encoding 488 aa for the S segment, 4,099 bp encoding 1,263 aa for the M segment, and 12,001 bp encoding 3,950 aa for the L segment. We performed multiple alignments using MAFFT version 7 (https://mafft.cbrc.jp/alignment/server) (9) and constructed a phylogenetic tree in MEGA7 (https://www.megasoftware.net) by using the neighbor-joining method with a bootstrap test for 1,000 replicates (10).

Phylogenetic analysis showed the strain belongs to the genus Orthonairovirus, family Nairoviridae, and is genetically related to SLV (Figure 2) (4,5,8,11). The terminal nucleotides of the S segment were identical to those of orthonairoviruses (3' segment terminus AGAGUUUCU and 5' segment terminus AGAAACUCU) (5). The termini of the M and L segments were different (Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/6/23-0056-App1.pdf). Homology analysis comparing YB_tick_2021_24 with SLV sample YC585 showed 71.9% nucleic acid (na) and 71.5% aa identities for the S segment, 72.4% na and 79.5% aa identities for the M segment, and 73.0% na and 84.6% aa identities for the L segment (Table 1) (8). Those results indicate that the isolate represents a unique Orthonairovirus species. For purposes of archiving, we designated novel YB_tick_2021_24 as Antu virus and deposited the strain in the National Pathogen Resource Center (accession no. NPRC 2.3.9401).

Conclusion

We identified a novel orthonairovirus, Antu virus, in *Dermacentor silvarum* ticks collected in China near the China-North Korea border. Nucleotide and amino acid sequence homologies, combined with phylogenetic analysis of other orthonairovirus genomes, suggested that Antu virus is a new member of the genus *Orthonairovirus*, genetically related to SLV. Tamdy virus and SLV are orthonairoviruses reportedly able to infect human and livestock (8,12,13). Lacking direct evidence of the ability of Antu virus to infect and cause illness among humans and livestock animals, we recommend enhanced monitoring and surveillance for Antu virus infection among humans and livestock in potentially endemic areas.

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About the Author

Dr. Fan Li is an associate professor at National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention. Her research interests include virus discovery in disease vectors and arbovirus infections.

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Address for correspondence: Huanyu Wang, National Institute for Viral Disease Control and Prevention, State Key Laboratory for Infectious Disease Prevention and Control, Chinese Center for Disease Control and Prevention, No.155 Changbai Rd, Changping District, Beijing 102206, China; email: wanghy@ivdc.chinacdc.cn; Qiang Wei, National Pathogen Resource Center, Chinese Center for Disease Control and Prevention, No.155 Changbai Rd, Changping District, Beijing 102206, China; email: weiqiang@chinacdc.cn

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Appendix

CCHFV-L TCTCAAAGAATATCATCCCCCCGTT— TCTCAAAGAAAACATTCTTGGGGACG CCHFV-M TCTCAAAGAAAACACGTGCCGCTTA TCTCAAAGAAAACACGTGCCGCTTA TCTCAAAGAAAACACGTGCCGCTTA TCTCAAAGAAAACCGTGCCGCTTA TCTCAAAGAAAACCGTGCCGCTTA TCTCAAAGAAAACCGTGCCGCTTA TCTCAAAGAAAAACCGTGCCGCTTA TCTCAAAGAAAAACCGTGCCGCACC GGTTTAGGGAACACTTTCTTTGAGA HUGV-M TCTCAAAGAAAGACCTGCAGCAAC TCTCAAAGAAAAACAAAAC		5' terminus	3' terminus
CCHFV-S TCTCAAAGAACACGTGCCGCTTA HUGV-L TCTCAAAGAACACGTGCCGCACCC GGTTTAGGGAACACTTTCTTTGAGA HUGV-M TCTCAAAGAACACGTGCCACCCCAC GTTAGCGGCAACGATATCTTTGAGA HUGV-S TCTCAAAGAACACGTGCCTATCC GTTAGCGGCAACAATCTTTGAGA HUGV-S TCTCAAAGAACGCATCCCCCCC TCTCAAGAACACACCCCCCCC TCTCAAAGAACACACTACCTTTGAGA GTATGCCGCTCCTATATCTTTGAGA ERVEV-M TCTCAAAGAACACACTACCGGCCAA GTATGCCGCTCCTATATCTTTGAGA BRVEV-S TCTCAAAGAACACTATCGTGCTACT GTATGCCGCTCCTATATCTTTGAGA TDV-L CACACCCAAATACATAGAACCAGG CCGGTGTTTATGGTATTTGGGTGT TDV-W CACACCCAACACTTTACACTTTAGG CCTTGAGCAATTTTGGTGTGT TV-L TCTCAAAGAACATATATCCTGCACAC GCTGGCACACAAAATCGGTGAAATCTTT TV-W CTGCAGCACCCAAAAGCCTTTCA GCCGTGAAAAACGGTGAAAATCACTTTCA TV-S AACGTGCTGCACACCCAAAAGCCTTTCA GTGACCCAACACTCTTCTTGGTGTAATT HTV-L AAGATATATATCCTGCACACCCAAA HTV-S CTGCACCCCAAAACCTTAAGAACCAACC ATATAAAGAGATCGGTGAAAAA HTV-S CTGCACCCCAAAACCTTAAAGCAAC TT-S AACGTGTGAAGGCAACCAAAAGCCTTTCA GTGACCCAACTCTTCTTGGTGTGAAA HTV-S CTGCACCCCAAAACCTTAAAGCAAC ATATAAAGAGATCGGTAGAAAAA HTV-S CTGCACCCCAAAACCTAAAGCCATCC ATATAAAGAGATCGGTGTGCTGGAACA WTV-W CCCTACTAAAGGCTAAAGGTTAGCG TGGGTTAGGGTGTTGCTGGAACA WTV-W CCCTACTAAAGGCTAAAGGTTAGCG GTGGGGAAAGGTTGGTGGTGGTGG GTGGGGAAGGTTGGTGGTGGTGGTGGTGGTGGTGGTGGTG	CCHFV-L	TCTCAAAGATATCAATCCCCCCGTT	CTCTTTCTTAGCTATATCTTTGAGA
HUGV-L TCTCAAAGATATAAATCCCTACACC GGTTTAGGGGAACACTTTCTTTGAGA HUGV-M TCTCAAAGAAAGACCTGCAGCAAC GTTAGCGGCCACAATATCTTTGAGA HUGV-S TCTCAAAGAAAGACAGCTGCCTATCC GTTAGCGGCAACAATATCTTTGAGA HUGV-S TCTCAAAGAAAGCAATCCCCCCA TAGGGGGGAATCATCTTTGAGA ERVEV-L TCTCAAAGAAAGCAATCCCCCCA TAGGGGGGAATCATCTTTGAGA ERVEV-M TCTCAAAGAAAGCAATCACCCCCA GTATGCCGCTCCTATATCTTTGAGA ERVEV-S TCTCAAAGAAAGCTTGTGCTGTTACT GTATGCAGCAACACACTATCTTTGAGA TDYV-L CACACCCAAATACATAGAACCAGG CCGGTGTTTATGGTATTTGGGTGTG TDY-M CACACCCAAACATTATAAACCAAGTT CCAGGTGGATAAGATTTCTTTGGGTGTG TDV-L TCTCAAAGATATATACCTGCACAC GCTGGCACAAAAACCGTTTCA GCCGTGAAAAAACGGTGAAAATCATTTCA GCCGTGAAAAAACAAAAGAAAAACAAATCACTTTCA TTV-S AACGTGCTGCACACCCAAAA HTV-L AAGATATATATCCTGCACACCCAAAA HTV-L AAGATATATATCCTGCACACCCAAAA HTV-S CTGCACACCCAAAACCTAAAGCAATC ATATAAAGAGATCGGGTGAACAA HTV-S CTGCACACCCAAAACCTAAAGCAACC ATATAAAGAGATCTGGGTGTACTC WTV-L CCTAACACCCACTTAACATCTGCCAA TTGGGTTTAGGGTGTATGTGGTG WTV-L CCTAACACCCACATAAGCATCC GTAGGGTAAGAGTTGGTGTGTGCTG WTV-L CCTAACACCCACTTAACATCTGCCAA TTGGGTTTAGGGTGTATGGTGTATGTGTGTGTGTGTG	CCHFV-M	TCTCAAAGAAATACTTGCGGCACG-	GCGTGCCGCCACTATATCTTTGAGA
HUGY-M TCTCAAAGAAAGACCTGCAGCAAC GTTAGCGGCCACAATACTTTTGAGA HUGY-S TCTCAAAGAAAGACGTGCCTATCC GTTAGCGGCCACAATACTTTTGAGA ERVEV-L TCTCAAAGAAAGACAATCCCCCCA TAGGGGGAAATACTATCTTTGAGA ERVEV-M TCTCAAAGAAAGACAATCCCCCCA GTATGCCGCTCCTATACTTTTGAGA ERVEV-S TCTCAAAGAAAGACTAGCGGCAA GTATGCCGCTCCTATACTTTTGAGA TDYV-L CACACCCAAACATTACATCGGCACA GTATGCAGCAACACTATCTTTGAGA TDYV-L CACACCCAAACATTACATCGGCGCAA GTATGCAGCAACACTATCTTTTGAGA TDYV-L CACACCCAAACATTAGAACCAGG CCGGTGTTTATGGTATTTGGGTGT TDYV-M CACACCCAAACTTTACACTTTAGG CCTTGAGCAAAATCGGTGAAATTGTTTTGGTGTG TTV-L TCTCAAAGATATATATCCTGCACAC GCTGGCACAAAATCGGTGAAATTGT TTV-M CTGCAGCACACCAAAAGCCTTTCA GCCGTGAAAAAACAGAAAACAAAAC	CCHFV-S	TCTCAAAGAAACACGTGCCGCTTA-	CTGTGCGGCAACGGTATCTTTGAGA
HUGY-S TCTCAAAGAAAGCGTGCCTATCC TCTCAAAGAAAGCAATCCCCCCA TAGGGGGAATACTATCTTTGAGA ERVEV-L TCTCAAAGAAAGCAATCCCCCCA TAGGGGGAATACTATCTTTGAGA ERVEV-M TCTCAAAGAAAGCTAGCGGCAA GTATGCCGCTCCTATATCTTTGAGA ERVEV-S TCTCAAAGAAAGCTAGCGGCAA GTATGCAGCAACACTATCTTTGAGA TDYV-L CACACCCAAACTTTACATCAGACCAGG CCGGTGTTTATGGTTATTTGGGTGTG TDYV-M CACACCCAAACTTTACAACCAAGTT CAGGTGGATAAGATTTCTTTGGTGTG TDYV-S CACACCCAAACTTTACACTTTAGG CCTTGAGCAATTTGCTTTTGGTGTG TTV-L TCTCAAAGATATATATCCTGCACAC GCTGGCACAAAATCGTTTACACTTTCA GCCGTGAAAAAGAAAAAAAAAA	HUGV-L	TCTCAAAGATATAAATCCCTACACC-	GGTTTAG G G A A C A C T T C T T T G A G A
ERVEV-L TCTCAAAGAAAGCAATCCCCCCA TCTCAAAGAAAGCAATCCCCCCCA TCTCAAAGAAAGCAATCCCCCCCA TCTCAAAGAAAGCAATCCCCCCCA TCTCAAAGAAAGCAATCCCCCCAA GTATGCCGCTCCTATATCTTTGAGA ERVEV-S TCTCAAAGAAAGCTTGTGCTGTTACT GTATGCCGCTCCTATATCTTTGAGA TDYV-L CACACCCAAATACATAGAACCAGG CCCGGTGTTTATGGTATTTGGGTGTG TDYV-M CACACCCAAACTTATAAACCAAGTT CAGGTGGATAAGATTTCTTTGGTGTG TTV-L TCTCAAAGATATATTCCTGCACAC GCTGGCACAAAATCGGTGAAAATCGGTGAAATTGT TTV-M CTGCAGCACCACCAAAAGCCTTTCA GCCGTGAAAAAAGAAAAAAAAAA	HUGV-M	TCTCAAAGAAAGACCTGCAGCAAC	GTTAGCTGCCACAATATCTTTGAGA
ERVEV-M TCTCAAAGAAAGACTAGCGGCAA ERVEV-S TCTCAAAGAAAGACTGTGCTGTTACT GTATGCCGCACCACACCTATATCTTTGAGA TDVV-L CACACCCAAATACATAGAACCAGG CCGGTGTTTATGGTATTTGGTGTG TDVV-M CACACCCAAAGCATTATAAACCAAGTT CAGGTGGATAAGATTTTTTGGTGTG TDVV-S CACACCCAAACTTTACACTTTAGG CCTTGAGCAATTTGCTTTTGGTGTG TTV-L TCTCAAAGATATATATCCTGCACAC GCTGGCACAAAAATCGGTGAAAATCATTTACACTTTCA TTV-S AACGTGCTGCACACCCAAAAGCCTTTCA GTGACCCAAAAAGAAAAAAAAAA	HUGV-S	TCTCAAAGAAAGACGTGCCTATCC	GTTAGCGGCAACAATA TCTTTGAGA
ERVEV-S TCTCAAAGAAAGTTGTGCTGTTACT CACACCCCAAATACATAGAACCAGG CCGGTGTTTATGGTATTTGGTGTG TDVV-M CACACCCAAAGCATTATAAACCAAGTT CAGGTGGATAAGATTTTTTGGTGTG TDVV-S CACACCCAAACTTTACACTTTAGG CCTTGAGCAATTTTGGTGTGT TTV-L TCTCAAAGATATATATCCTGCACAC GCCGGGACACAAAATCGGTGAAATCATTTTCA TTV-S AACGTGCTGCACACCCAAAAGCCTTTCA TTV-S AACGTGCTGCACACCCAAAAGCCTTTCA HTV-L AAGATATATATCCTGCACACCCAAA HTV-M AAACAGTGTGAAGGCAATGATGAG AAACAAACAAAAGAAAAAAAAAA	ERVEV-L	TCTCAAAGAAAGCAATCCCCCCA	TAGGGGGGAATACTATCTTTGAGA
TDYV-L CACACCCAAATACATAGAACCAGG TDYV-M CACACCCAAGCATTATAAACCAAGTT CAGGTGGATAAGATTTCTTGGGTGTG TDYV-S CACACCCCAAACTTTACACTTTAGG CCTTGAGCAATTTGCTTTTGGGTGTG TTV-L TCTCAAAGATATATATCCTGCACAC GCTGGCACAAAAACGAAAAACGAAATACA TTV-S AACGTGCTGCACACCAAAAGCCTTTCA GTGACCAACTTTACACTTTGGTTGTAAGTT HTV-L AAGATATATATCCTGCACACCAAAA GTGACCAACTCTTCTTTGGTGTAAAAAAAAAA	ERVEV-M	TCTCAAAGAAAGACTAGCGGCAA-	GTATGCCGCTCCTATA TCTTTGAGA
TDYV-M CACACCCAAGCATTATAAACCAAGTT TOV-S CACACCCCAAACTTTACACTTTAGG CCTTGAGCAATTTGCTTTGGTGTG TTV-L TCTCAAAGATATATATCCTGCACAC GCTGGCACAAAAATCGGTGAAAATCATTTCA GCCGTGAAAAAAAAAA	ERVEV-S	TCTCAAAGA AAGTTGTGCTGTTACT-	GTATG CAGCAACACTA TCTTTGAGA
TDYV-S CACACCCAAACTITACACTITAGG TTV-L TCTCAAAGATATATATCCTGCACAC GCTGGCACAAAATCGGTGAAATTGT TTV-M CTGCAGCACACCAAAAGCCTTTCA GCCGTGAAAAAGAAAAG	TDYV-L	CACACCCA AATACATAGAACCAGG-	CCGGTGTTTATGGTATTTGGGTGTG
TTV-L TCTCAAAGATATATATCCTGCACAC GCTGGCACAAAATCGGTGAAAATTGT TTV-M CTGCAGCACACCAAAAGCCTTTCA GCCGTGAAAAAGAAAAG	TDYV-M	CACACCAAGCATTATAAACCAAGTT	CAGGTGGATAAGATTTCTTGGTGTG
TTV-M CTGCAGCACACCAAAAGCCTTTCA————————————————	TDYV-S	CACACCCAAACTTTACACTTTAGG-	
TTV-S AACGTGCTGCACACCAATAGCATTC ATCAGTTTACTACTGGTGTAAGTT HTV-L AAGATATATACCTGCACACCCAAA GTGACCAACCAACAAAAAAAAAA	TTV-L	TCTCAAAGATATATCCTGCACAC-	GCTGGCACAAATCGGTGAAATTGT
HTV-L AAGATATATATCCTGCACACCCAAA HTV-M AAACAGTGTGAAGGCAATGATGAG —AAACAAACAAAAGAGAACGTCTCTTGGTCTGATA HTV-S CTGCACACCAAAACCTAAAGCAAC —ATATAAAGAGATCGGAAGAGCGTCG WTV-L CCCTAACACCACCTTAACATCTGCCAA —TAGGGTTARGGTGTAGTGGTGCTG WTV-S ATCACCTACATCGAATACCCATCCC GTAAGTTAAAGGGTGTGCAGCAACA SGLV-L TCTCAAAGATATATATCCTGCACA GTGTGGGGGAAGGTTGATCCCATGT SGLV-M ACATGGGATAGTAACCTGTGCTAG —CCTGTCTCGACCATGCCCCCCCATGT SGLV-S TCTCAAAGAAAACACACCCCCCCAAAC ATV-L GATATATATCCTGCACACCCAAAC —AGGGTCCCGCCTCTGCAACTGCCCC ATV-M TGTCAGCATCGAAGGAGGGAACA —AAGGTCCCGCCTCTGCAACTGCCCC ATV-M AGGGTCCCGCCTCTGCAACTGCCCC —AAGGTCCCGCCTCTGCAACTGCCCCCAACC —AAGGTCCCGCCTCTGCAACTGCCCCCCCCCCCCCCCCCC	TTV-M	CTGCAGCACCAAAAGCCTTTCA	GCCGTGAAAAGAAAGAAATACA
HTV-M AAACAGTGTGAAGGCAATGATGAG —AAACAAACAAAAGAGAAAAAA HTV-S CTGCACACCAAAACCTAAAGCAAC —ATATAAAGAGATCGGAAGAGCGTCG WTV-L CCTAACACCACTTAACATCTGCCAA —TAGGGTTARGGTGTAGGGAACA WTV-M CCCTACTAAAGGCTAAAGGTTAGCG —TGGTGTTAAGGTGATTAGGTGTGCTG WTV-S ATCACCTACATCGAATACCCATCCC —GTAAGTTAAAGGGTGGCAGCAACA SGLV-L TCTCAAAGATATATATCCTGCACA —GTGTGGGGAAGGTTGATCCCATGT SGLV-M ACATGGGATAGTAACCTGTGCTAG —CCTGTCTCGACCATGCCCCCCATGT ATV-L GATATATATCCTGCACACCCAAAC —AGGTCCCGCCTCTGCAACTGCCCC ATV-M TGTCAGCATCGAAGGAGGGGAACA —AAGGTCCCGCCTCTGCAACTGCCC ATV-M TGTCAGCATCGAAGGAGGGGAACA —AAGGTCCCGCCTCTGCAACTGCCCC ATV-M TGTCAGCATCGAAGGAGGGGAACA —AAGGTCCCGCCTCTGCAACTGCCCC ATV-M ACATGAAACAAAACAAATCCCCCTAACC —AAGGTCCCGCCTCTGCAACTGCCCC ATV-M AAGGTCCCGCCTCTGCAACTGCCCC	TTV-S	AACGTGCTGCACACCAATAGCATTC-	ATCAGTTTACTACTGGTGTAAGTT
HTV-S CTGCACCCAAAACCTAAAGCAAC WTV-L CCTAACACCCACTTAACATCTGCCAA WTV-M CCCTACTAAAGGCTAAAGGTTAGCG WTV-S ATCACCTACATCGAATACCCATCCC GTAAGTTAAAGGGTGTGCAGCAACA SGLV-L TCTCAAAGATATATATCCTGCACA GTGTGGGGAAGGTTGATCCCATGT SGLV-M ACATGGGATAGTAAACCTGTGCTAG CCTGTCTCGACCATGCCCCCCATGT ATV-L GATATATATCCTGCACACCCAAAC AGGGTCCCGCCTCTGCAACCTCCC AGGGTCCCGCCCTCTGCAACCTCCCCCCCCCC	HTV-L	AAGATATATCCTGCACACCCAAA	————GTGACCAACTCTTCTTGGTCTGATA
WTV-L CCTAACACCACTTAACATCTGCCAA WTV-M CCCTACTAAAGGCTAAAGGTTAGCG WTV-S ATCACCTACATCGAATACCCATCCC GTAAGTTAAAGGTGTGCAGCAACA GGUV-L TCTCAAAGGATATATATCCTGCACA GGUV-S TCTCAAAGAAAAACAAAACCTGTGCTAG CCTGTCTCGACCATGCCCCCCCATGT AGAAACAAAAACAAAATTCCCCCATGT ATV-L GATATATATCCTGCACCACCCAAAC AGCTGCTGTGGAGATCTGGTGACCT ATV-M TGTCAGCATCGAAGGAGGGAACA AAGGTCCCGCCTCTGCAACTGCCC AAGGTCCCGCCTCTGCAACTGCCCCCAACC AAGGTCCCGCCTCTGCAACTGCCCCCAACC AAGGTCCCGCCTCTGCAACTGCCCCCAACC AAGGTCCCCGCCTCTGCAACTGCCCCCCCCCC	HTV-M	AAACAGTGTGAAGGCAATGATGAG-	AAACAAACAAAGAGAAAAA
WTV-M CCCTACTAAAGGCTAAAGGTTAGCG —TGGTGTTAGGTGTATTGGTGCTG WTV-S ATCACCTACATCGAATACCCATCCC —GTAAGTTAAAGGGTGTGCAGCACAC SGLV-L TCTCAAAGATATATATCCTGCACA —CCTGTCTCGACCATGCCCCCCATGT SGLV-S TCTCAAAGAAAACCTGTGCTAG —AGAAACAAAACAAATTCCCCCATGT ATV-L GATATATATCCTGCACACCCCAAAC —AGCTGCTGTGGAGTCTGGTGACCT ATV-M TGTCAGCATCGAAGGAGGGAACA —AAGGTCCCGCCTCTGCAACTGCCCC ATV-M ACATGGTATATATCCTGCACACCCAAAC —AAGGTCCCGCCTCTGCAACTGCCC	HTV-S	CTGCACACCAAACCTAAAGCAAC	ATATAAAGAGATCGGAAGAGCGTCG
WTV-S ATCACCTACATCGAATACCCATCCC GTAAGTTAAAGGGTGTGCAGCAACA SGLV-L TCTCAAAGATATATATCCTGCACA SGLV-M ACATGGGATAGTAACCTGTGCTAG CCTGTCTCGACCATGCCCCCCATGT SGLV-S TCTCAAAGAAAACACACCCCACACC AGAAACAAAACA	WTV-L	CCTAACACCACTTAACATCTGCCAA-	TAGGGTTARGGGTGTGCAGGAACA
SGLV-L TCTCAAAGATATATCCTGCACA GTGTGGGGAAGGTTGATCCCATGT SGLV-M ACATGGGATAGTAACCTGTGCTAG CCTGTCTCGACCATGCCCCCCCATGT SGLV-S TCTCAAAGAAAACGTGCTGCACACC AGAAACAAAACAA	WTV-M	CCCTACTAAAGGCTAAAGGTTAGCG-	TGGTGTTAGGTGTATTGGTGTGCTG
SGLV-M ACATGGGATAGTAACCTGTGCTAG — CCTGTCTCGACCATGCCCCCCATGT SGLV-S TCTCAAAGAAAACGTGCTGCACACC — AGAAACAAAACAAATTCCCCCATGT ATV-L GATATATATCCTGCACACCCCAAAC — AGCTGCTGTGGAGTCTGGTGACCT ATV-M TGTCAGCATCGAAGGAGGGAACA — AAGGTCCCGCCTCTGCAACTGCCT	WTV-S	ATCACCTACATCGAATACCCATCCC-	GTAAGTTAAAGGGTGTGCAGCAACA
SGLV-S TCTCAAAGAAAACGTGCTGCACAC AGAAACAAAACAAA	SGLV-L	TCTCAAAGATATATCCTGCACA-	GTGTGGGGAAGGTTGA <mark>TCCCATGT</mark>
ATV-L GATATATCCTGCACACCCAAAC — AGCTGCTGTGGAGTCTGGTGACCT ATV-M TGTCAGCATCGAAGGAGGGAACA — AAGGTCCCGCCTCTGCAACTGCCT	SGLV-M	ACATGGGATAGTAACCTGTGCTAG-	CCTGTCTCGACCATGCCCCCCATGT
ATV-M TGTCAGCATCGAAGGAGGGAACA —————————————————————————	SGLV-S	TCTCAAAGAAACGTGCTGCACAC	AGAAACAAAACAAATTCCCCATGT
	ATV-L	GATATATCCTGCACACCCAAAC	AGCTGCTGTGGAGTCTGGTGACCT
ATV-S TCTCAAAGAAAAACGTGCTGCACA — GTGCAGCAACAATATCTTTGAGA	ATV-M	TGTCAGCATCGAAGGAGGGAACA	AAGGTCCCGCCTCTGCAACTGCCT
	ATV-S	TCTCAAAGAAAACGTGCTGCACA —	GTGCAGCAACAATA TCTTTGAGA

Appendix Figure. Terminal sequences of ATV and other orthonairoviruses. Typical terminal sequences of orthonairoviruses are highlighted in yellow and putative terminal sequences in red. L, Large segment;

M, medium segment; S, small segment; CCHFV, Crimean-Congo hemorrhagic fever virus (L, NC_005301; M, NC_005300; S, NC_005302); HUGV, Hughes virus (L, NC_040512; M, NC_040513; S, NC_040514); ERVEV, Erve virus (L, JF911697; M, JF911698; S, JF911699); TDYV, Tamdy virus (L, MK757580; M, MK757581; S, MK757582); TTV, Tacheng tick virus 1 (L, NC_031284; M, NC_031285; S, NC_031286); HTV, Huangpi tick virus 1 (L, NC_031135; M, NC_031136; S, NC_031137); WTV, Wenzhou tick virus (L, NC_031291; M, NC_031288; S, NC_031289); SGLV, Songling virus (L, MT328776; M, MT328775; S, MT328777); ATV, Antu virus.