





Terminal Genome Sequences of the Soft Tick Bunyavirus

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Satoko Sugimoto, Yuto Suda, and Tomoki Yoshikawa contributed equally to this work. Yuto Suda was the first person to establish the methods for preparation and titration of STBV and ISKV, which were manipulated for RNA preparation in the study. Satoko Sugimoto determined full genome sequences of the two viruses and prepared the manuscript. Tomoki Yoshikawa helped Satoko Sugimoto to determine the terminal sequences of the viruses using the RACE method. The order in which they are listed was determined by the concept's significance to the manuscript; the three authors made indispensable contributions to the manuscript.

ABSTRACT The complete genome sequence of the soft tick bunyavirus (STBV) was obtained using the Sanger sequencing technique. Comparison with other viral sequences revealed that STBV has unique sequences in the terminal regions that are highly conserved among the genus *Orthonairovirus*.

The soft tick bunyavirus (STBV) was originally isolated by Oba et al. (1) from a pool of soft ticks, *Argas vespertilionis*, collected in feces beneath bat colonies located in a human habitat in Japan (2). A next-generation sequencing analysis revealed that the STBV genome consists of three negative-strand RNA segments (1). The virus is most closely related to Keterah virus isolated in Malaysia (3), which is a member of the species *Keterah orthonairovirus* of the genus *Orthonairovirus* of the family *Nairoviridae*. The sequence of STBV deposited in GenBank (accession no. [LC027465](#) to [LC027467](#)) lacked the 3' and 5' terminal sequences in each segment (24 and 20 nucleotides in segment L, 24 and 17 nucleotides in segment M, and 228 and 23 nucleotides in segment S at the 3' and 5' termini, respectively). Previously, it was recognized that each segment of *Orthonairovirus* genomes has nine complementary terminal consensus sequences, 3' terminus AGAGUUUCU and 5' terminus AGAAACUCU (4). However, Kuhn et al. (3) reported that several recently identified *Orthonairovirus* members have consensus regions that differ by one or two nucleotides. Here, we determined the complete genome sequence, including the terminal regions of STBV, and compared the terminal sequences with those of other *Orthonairovirus* members.

STBV was passaged three times in Vero cells and once in BME/CTVM2 cells (5). The viral RNA was extracted from the culture supernatant using a High Pure viral RNA kit (Roche Applied Science, Mannheim, Germany). To obtain DNA corresponding to the viral terminal regions, rapid amplification of cDNA ends (RACE) (6) was performed. Virus-specific primers used in RACE were as follows: CCCAGTAATCATCTCTC for the L segment, TCTCTGTGCTCACTGTC for the M segment, and GAAGCAGAGAGATTGCT for the S segment at the 3' termini and GGACTAATCTAATCTGCGGC for the L segment, GGAACACCGCAGTACTATCT for the M segment, and GGCTTCTACCTGCACTAACA for the S segment at the 5' termini. The amplified products were directly used for sequencing without cloning using Sanger's method (7) with an Applied Biosystems 3500XL genetic analyzer (Thermo Fisher Scientific) according to the manufacturer's protocol. The complete sequences of the STBV L, M, and S segments were deposited in GenBank under accession no. [LC495731](#), [LC495732](#), and [LC495733](#), respectively.

Although a few differences were detected in the L segment (3 nucleotides) and S segment (13 nucleotides), the determined sequences were almost the same as those in

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TABLE 1 The nine terminal nucleotide sequences of viral genomes in the genus *Orthonaïrovirus*^a

Sequence for ^b :											
Species	Virus	L segment			M segment			S segment			Reference or accession no. ^d
		3'	5'		3'	5'		3'	5'		
	Consensus sequence	AGAGUUUCU	AGAAACUCU		AGAGUUUCU	AGAAACUCU		AGAGUUUCU	AGAAACUCU		4
Keterah	Keterah virus	NA	NA		AGAGUUUCU	NA		NA	NA		KR537447, KR537448, KR537449
Keterah	Soft tick bunyavirus ^c	AGAGUUUCU	AGAAUCUCU		AGAGUUUCU	AGAAUCUCU		AGAGAUUCU	AGAAUCUCU		LC495731, LC495732, LC495733
Keterah	Issyk-Kul virus ^c	AGAGUUUCU	AGAAUCUCU		AGAGUUUCU	AGAAUCUCU		AGAGAUUCU	AGAAUCUCU		KR709221, KR709220, KR709219
Qalyub	Qalyub virus	AGAGAUUCU	AGAAACUCU		AGAGAUUCU	NA		AGAGAUUCU	AGAAUCUCU		KU925476, KU925477, KU925478
Dera Ghazi Khan	Dera Ghazi Khan virus	AGAGUUUCU	AGAAACUCU		AGAGUUUC	AGAAACUCU		AGAGUUUC	AGAAACUCU		KU925452, KU925453, KU925454
Dera Ghazi Khan	Abu Hammad virus	AGAGUUUC	UGAAACUCU		AGAGUUUC	UGAAACUCU		AGAGAUUC	UGAAACUCU		KU925434, KU925435, KU925436
Dera Ghazi Khan	Abu Mina virus	AGAGUUUC	UGAAACUCU		AGAGUUUC	UGAAACUCU		AGAGAUUC	UGAAACUCU		KU925437, KU925438, KU925439
Dera Ghazi Khan	Tunis virus ^c	AGAGUUUC	UGAAACUCU		AGAGUUUC	UGAAACUCU		AGAGAUUC	UGAAACUCU		KU925497, KU925498, KU925499
Dera Ghazi Khan	Sapphire II virus ^c	AGAGUUUC	UGAAACUCU		AGAGUUUC	UGAAACUCU		AGAGUUUC	UGAAACUCU		KU925485, KU925486, KU925487

^a Shown are members of the genus *Orthonaïrovirus* whose terminal nine nucleotides in their genomes are different from the consensus sequences (4).

^b NA, not available; bold, different bases from the consensus; underline, noncomplementary nucleotide pairs.

^c Proposed classification by Kuhn et al. (3).

^d Accession numbers are given for the L, M, and S segments, respectively.

the previously reported sequences, except for the termini (1). The terminal 9-nucleotide sequences of STBV were not the same as those of the consensus sequences of the genus *Orthonairovirus* (4), and noncomplementary nucleotide pairings at the fifth-most-terminal nucleotide were observed in the L and M segments (Table 1). We observed the same terminal sequences in the Issyk-Kul virus (ISKV) propagated in SW-13 cells by applying the strategy for sequencing described above (GenBank accession no. [LC495734](#) to [LC495736](#)); terminal nucleotides of ISKV were identical to those reported by Atkinson et al. using the virus propagated in a suckling mouse (8). Thus, the terminal sequence appears to well reflect the reported phylogenetic tree (3). Similar terminal sequences with one or two mismatched pairings were observed for STBV-related viruses, e.g., Qalyub virus and Dera Ghazi Khan virus (Table 1).

In summary, a full-genome sequence, including the termini of STBV was determined using Sanger's method. The STBV has the same terminal sequences as ISKV. Determination of terminal sequences of viral genomes might accelerate the classification of *Orthonairovirus* members.

Data availability. The sequences determined in the present study have been deposited under the accession no. [LC495731](#), [LC495732](#), and [LC495733](#) (STBV) and [LC495734](#), [LC495735](#), and [LC495736](#) (ISKV).

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