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Sequence and phylogenetic analysis of the large (L) segment of the Tahyna virus genome

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Abstract The Tahyna virus (TAHV) is an important human pathogen in the *Bunyaviridae* family. To date, only the S and M segments of this virus have been sequenced, but the sequence of the L segment hasn't been established yet. In this study, we sequenced 963 nucleotides of the L segment of TAHV, comprising pre-motif A and motif A in region 3 of the RNA polymerase gene.

The nucleotide sequence data reported in this paper was submitted to the GenBank nucleotide sequence database: EU185046.

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Tahyna virus (TAHV) is a member of the California serogroup of the genus *Orthobunyavirus* in the *Bunyaviridae* family that causes a febrile illness and sometimes leads to meningoencephalitis and atypical pneumonia [1–3]. Although no fatal cases have been reported, the World Health Organization (WHO) warns that TAHV must be considered important for public health, since it and its vectors are widespread in Europe. Moreover, TAHV possesses a great emergent potential, as it is capable of causing severe disease [3].

To date, only the S and M segments of TAHV have been analyzed [1, 2, 4]. Here, we determine the nucleotide (nt) and deduced amino acid (aa) sequences of region 3 of the L segment of TAHV and compare it with other members of the *Bunyaviridae* family.

Virus propagation, RNA extraction, reverse transcription, cloning, and sequencing were done as described previously [5]. Primers used were LAC-L2345F (5′- GTC ATG GTGGATCTAGCAAAGAC - 3′) and LAC-L3285R (5′- GGGTCTAAGG CTATGAGCC - 3′).

The 963 bp nucleotide (nt) sequence obtained for the L segment of TAHV was compared to sequences of other members of the *Orthobunyavirus* genus, such as the Akabane (AKAV AB190458), Caraparu (CARV EF122411), Bunyamwera (BUNV NC001925), Oropouche (OROV NC005776), and La Crosse (LACV AF525489) viruses. When compared to these other viral sequences, the L segment of TAHV showed nucleotide and deduced amino acid sequence identities ranging from 61.9% to 76.8% and 54.5% to 90.0%, respectively. It is important to highlight

the higher identities (76.8 and 90.0%) observed between TAHV and LACV, which correlate with the fact that both viruses belong to the same serogroup (California). In the phylogenetic tree (Fig. 1a), TAHV clustered with the *Orthobunyavirus* genus and with the California Encephalitis serogroup (which is represented by the LACV), confirming its serological classification. Moreover, TAHV and LACV are more closely related to BUNV than CARV, OROV, or AKAV.

The L segment sequence described here corresponds to part of region 3, which is one of the four conserved regions shared in the RNA polymerase of the *Bunyaviridae* family members. Region 3 is located in the middle of the L segment, which comprises the pre-motif A and the motifs A, B, C, D, and E, present in almost all RNA-dependent polymerases [6–9]. Here, we report the sequence of pre-motif A and motif A (Fig. 1b).

In pre-motif A, TAHV presents three strictly conserved amino acids (Lys-950, Arg-958, and Glu-975) and one strongly conserved amino acid (Lys-968), which was previously reported by Muller et al. [9] for negative-stranded RNA virus polymerases. A strictly conserved residue

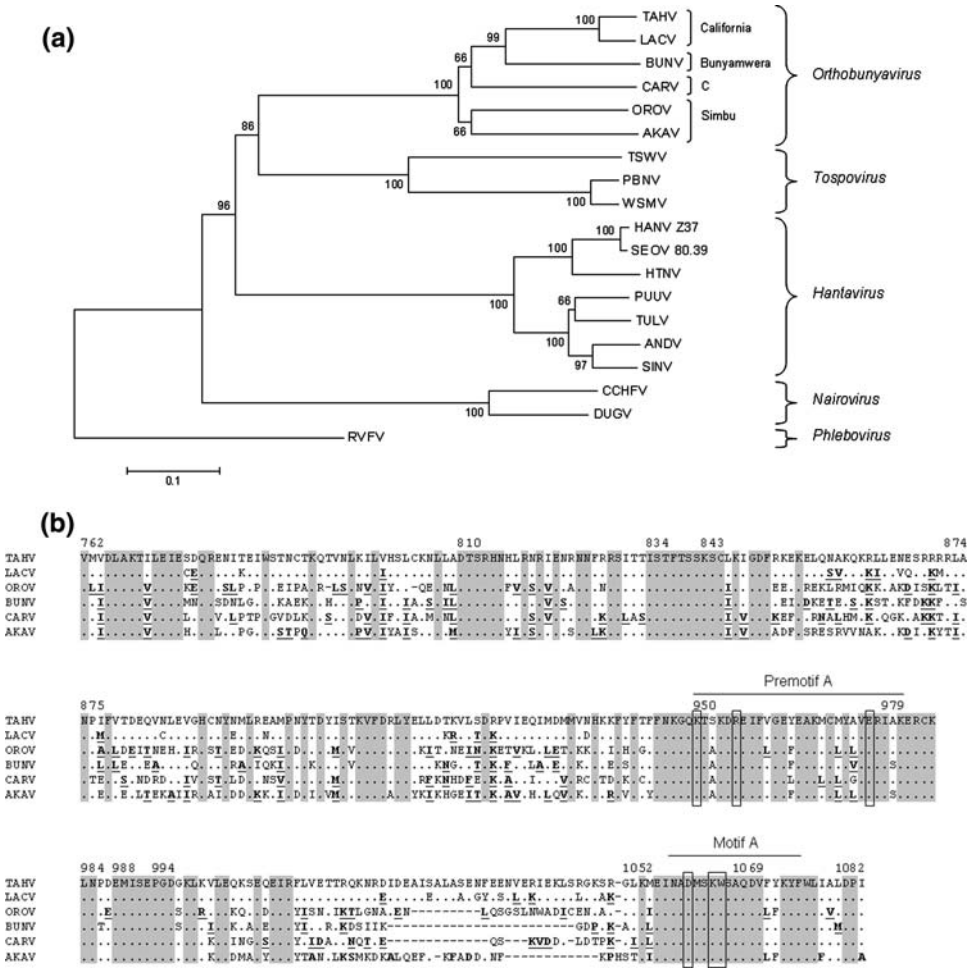

Fig. 1 Phylogenetic tree and alignment of the deduced 321 aa sequence of the TAHV L segment and other members of the *Orthobunyavirus* genus. (**a**) Phylogenetic tree was generated by NJ analysis using the p-distance. Numbers adjacent to each branch represent the bootstrap values obtained with 1,000 replicates. Rift Valley Fever Virus (RVFV), a member of the genus *Phlebovirus*, was used as an outgroup to root the tree. The scale bar represents 0.1% amino acid sequence divergence. The following published L segment sequences were used: La Crosse (LACV, AF525489), Bunyamwera (BUNV, NC001925), Caraparu (CARV EF122411), Oropouche (OROV, NC005776), Akabane (AKAV, AB190458), Tomato spotted wilt (TSWV, AB198742), Peanut bud necrosis (PBNV, AF025538), Watermelon silver mottle (WSMV, AF133128), Hantavirus Z37 (HANV Z37, AF285266), Seoul 80.39 (SEOV 80.39, X56492), Hantaan (HTNV, AF336826), Puumala (PUUV, M63194), Tula (TULV, NC005226), Andes (ANDV, NC003468), Sin Nombre (SINV, NC005217), Crimean-Congo hemorrhagic fever (CCHFV, NC005301), Dugbe (DUGV, NC004159) and Rift Valley fever (RVFV, NC002043). (**b**) Pre-motif A and motif A are indicated. Dots represent amino acid identity with the TAHV sequence. Residues strictly conserved among all genera in the *Bunyaviridae* are boxed. Bold-faced and underlined characters indicate the amino acids chemically similar to those of TAHV. Amino acids conserved among the members of *Orthobunyavirus* are labeled in gray. The numbers correspond to the position of amino acids in the LACV L segment

(Asp-1060), which is present in all investigated RNA-dependent polymerases, is also present in TAHV motif A, which reflects its crucial importance for RNA template recognition and/or polymerase activity [8].

In summary, six strictly conserved amino acids were observed within these two blocks (pre-motif A and motif A) for *Bunyaviridae* family members. Several other conserved regions were also observed across members of *Orthobunyavirus*. When we compared pre-motif A and motif A (321 aa) of TAHV to that of LACV, we observed 32 amino acid differences. However, 15 out of these 32 aa are conservative substitutions, revealing a high identity between the two sequences and, indeed, confirming their placement in the same serogroup (Fig. 1b).

Our data indicate that the RNA polymerase of TAHV has the same basic structure as other RNA polymerases of the *Bunyaviridae*. The completion of the TAHV genome sequence will certainly contribute to a better understanding of the biology of this virus.

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