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Diversity among Tacaribe serocomplex viruses (family *Arenaviridae*) naturally associated with the Mexican woodrat (*Neotoma mexicana*)

Maria N. B. Cajimat^{a,1}, Mary Louise Milazzo^b, Jeff N. Borchert^{c,2}, Ken D. Abbott^d, Robert D. Bradley^e, and Charles F. Fulhorst^{b,*}

^a Microbiology and Immunology Graduate Program, Graduate School of Biomedical Sciences, University of Texas Medical Branch, Galveston, TX 77555-1019, USA

^b Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555-0609, USA, mamilazz@utmb.edu and cfulhors@utmb.edu

^c Genesis Laboratories, Inc., Wellington, CO 80549, USA

^d Department of Biology, Yavapai College, Prescott, AZ 86301, USA, kenabbott@cableone.net

^e Department of Biological Sciences and Museum, Texas Tech University, Lubbock, TX 79409-3131, USA, robert.bradley@ttu.edu

Abstract

The results of analyses of glycoprotein precursor and nucleocapsid protein gene sequences indicated that an arenavirus isolated from a Mexican woodrat (*Neotoma mexicana*) captured in Arizona is a strain of a novel species (proposed name *Skinner Tank virus*) and that arenaviruses isolated from Mexican woodrats captured in Colorado, New Mexico, and Utah are strains of *Whitewater Arroyo virus* or species phylogenetically closely related to *Whitewater Arroyo virus*. Pairwise comparisons of glycoprotein precursor sequences and nucleocapsid protein sequences revealed a high level of divergence among the viruses isolated from the Mexican woodrats captured in Colorado, New Mexico, and Utah and the *Whitewater Arroyo virus* prototype strain AV 9310135, which originally was isolated from a white-throated woodrat (*Neotoma albigula*) captured in New Mexico. Conceptually, the viruses from Colorado, New Mexico, and Utah and strain AV 9310135 could be grouped together in a species complex in the family *Arenaviridae*, genus *Arenavirus*.

Keywords

Arenaviridae; *Neotoma mexicana*; Mexican woodrat; *Skinner Tank virus*; *Whitewater Arroyo virus*; arenavirus species complex

* Correspondence to -- Charles F. Fulhorst at University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0609, USA, Tel 409.772.9713, Fax 409-747-2437, cfulhors@utmb.edu.

¹Current affiliation: Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555-0609, USA, nbcajirm@utmb.edu

²Current affiliation: Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO 80521, USA, jborchert71@hotmail.com

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1. Introduction

The virus family *Arenaviridae*, genus *Arenavirus* comprises 2 serocomplexes and 22 species (Salvato et al., 2005). The lymphocytic choriomeningitis-Lassa (Old World) serocomplex includes *Lassa virus* (LASV), *lymphocytic choriomeningitis virus* (LCMV), *Ippya virus* (IPPV), *Mobala virus* (MOBV), and *Mopeia virus* (MOPV). The Tacaribe (New World) serocomplex includes *Bear Canyon virus* (BCNV), *Tamiami virus* (TAMV), and *Whitewater Arroyo virus* (WWAV) in North America, *Tacaribe virus* (TCRV) on Trinidad in the Caribbean Sea, and *Allpahuayo virus* (ALLV), *Amapari virus* (AMAV), *Cupixi virus* (CPXV), *Flexal virus* (FLEV), *Guanarito virus* (GTOV), *Junín virus* (JUNV), *Latino virus* (LATV), *Machupo virus* (MACV), *Oliveros virus* (OLVV), *Paraná virus* (PARV), *Pichindé virus* (PICV), *Piritai virus* (PIRV), and *Sabiá virus* (SABV) in South America. Provisional members of the Tacaribe serocomplex include *Catarina virus* (CTNV) (Cajimat et al., 2007).

The genomes of arenaviruses comprise 2 single-stranded RNA segments, designated large (L) and small (S) (Salvato et al., 2005). The L segment (~ 7.5 kb) consists of a 5' non-coding region (NCR), the Z gene, an intergenic region that separates the Z gene from the RNA-dependent RNA polymerase (RdRp) gene, the RdRp gene, and a 3' NCR. Similarly, the S segment (~ 3.5 kb) consists of a 5' NCR, the glycoprotein precursor (GP-C) gene, an intergenic region that separates the GP-C gene from the nucleocapsid (N) protein gene, the N protein gene, and a 3' NCR. Our most comprehensive knowledge of the phylogenetic history of the Tacaribe serocomplex viruses is based on the results of analyses of full-length GP-C sequences and full-length N protein sequences (Archer and Rico-Hesse, 2002; Charrel et al., 2002).

Specific members of the rodent family Cricetidae (Musser and Carleton, 2005) are the principal hosts of the Tacaribe serocomplex viruses for which natural host relationships have been well characterized (Childs and Peters, 1993). For example, the southern plains woodrat (*Neotoma micropus*) in southern Texas is the principal host of CTNV (Cajimat et al., 2007; Fulhorst et al., 2002), the hispid cotton rat (*Sigmodon hispidus*) in southern Florida is the principal host of TAMV (Calisher et al., 1970; Jennings et al., 1970), and the drylands vesper mouse (*Calomys musculinus*) in central Argentina is the principal host of JUNV (Mills et al., 1992).

The WWAV prototype strain AV 9310135 originally was isolated from a white-throated woodrat (*Neotoma albigula*) captured in 1993 in McKinley County in northwestern New Mexico (Fulhorst et al., 1996). Subsequently, antibody to strain AV 9310135 was found in Mexican woodrats (*Neotoma mexicana*) captured in northern Arizona (Abbott et al., 2004) and southeastern Colorado (Calisher et al., 2001) and arenaviruses phylogenetically closely related to strain AV 9310135 were isolated from Mexican woodrats captured in southeastern Utah and central New Mexico (Fulhorst et al., 2001). The objective of this study was to increase our knowledge of the geographical range and genetic diversity of arenaviruses naturally associated with the Mexican woodrat (*N. mexicana*) in the western United States.

2. Materials and methods

Four arenaviruses were genetically characterized in this study: AV 96010024, AV 96010151, AV D1000090, and AV D1240007 (Table 1). AV 96010024 and AV 96010151 were isolated from Mexican woodrats NK36282 and NK62425 in a previous study (Fulhorst et al., 2001). The species identities of NK36282 and NK62425 were confirmed by comparisons of the nucleotide sequences of the cytochrome *b* genes of these rodents to the nucleotide sequences of the cytochrome *b* genes of other woodrats (Edwards and Bradley, 2002). AV D1000090 and AV D1240007 were isolated from Mexican woodrats TK119202 and TK123380 in this study. The species identities of these woodrats were confirmed by R. D. Bradley, using dental characteristics (Hall, 1981).

TK119202 and 38 other Mexican woodrats were captured in a 3-day period in August 2002 at a site near Skinner Tank in Coconino County, Arizona (Abbott et al., 2004) (Figure 1). Samples of spleen, kidney, and urine for virus assay were collected from TK119202, samples of spleen and kidney for virus assay were collected from 31 of the 38 other Mexican woodrats, and samples of heart, kidney, liver, lung, and skeletal muscle, and the skins and skeletons of all the woodrats were deposited into the Museum of Texas Tech University. Two of the 39 Mexican woodrats captured near Skinner Tank were antibody-positive to WWAV strain AV 9310135 (C. F. Fulhorst, unpublished data).

TK123380 and 18 other Mexican woodrats were captured in a 4-day period in October 2002 at a site near Big Thompson Canyon in northern Colorado (Figure 1). These woodrats were intended to be founders of a captive breeding colony at Genesis Laboratories, Inc. (Wellington, Colorado). Antibody to WWAV strain AV 9310135 was found in TK123380 and 3 of the 18 other woodrats in blood samples collected 2 weeks after capture (J. N. Borchert and M. L. Milazzo, unpublished data). The 4 antibody-positive animals were killed 4 weeks after capture and shipped on dry ice to the University of Texas Medical Branch, Galveston. Samples of spleen, kidney, and urine for virus assay were collected from the carcasses and then the carcasses were deposited into the Museum of Texas Tech University.

The samples of spleen, kidney, and urine from TK119202, spleen and kidney from 31 of the 38 other Mexican woodrats captured near Skinner Tank, and spleen, kidney, and urine from TK123380 and the 3 other antibody-positive woodrats captured near Big Thompson Canyon were tested for arenavirus as described previously (Fulhorst et al., 1996). Briefly, crude 10% w/v suspensions of the samples of spleen and kidney and 10% v/v suspensions of the samples of urine were inoculated onto monolayers of Vero E6 cells grown in 12.5-cm² plastic culture flasks, the inoculated monolayers were maintained under a fluid overlay at 37°C for 13 or 14 days, and then arenaviral antigen in infected Vero E6 cells was revealed by using an indirect fluorescent antibody test. The primary and secondary antibodies in the fluorescent antibody test were a hyperimmune mouse ascitic fluid raised against WWAV strain AV 9310135 and a goat anti-mouse IgG fluorescein conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD), respectively.

The nucleotide sequences of a 3,280- to 3,308-nt fragment of the S genomic segments of AV 96010024, AV 96010151, AV D1000090, and AV D1240007 were determined to establish the phylogenetic and taxonomical relationships between these arenaviruses, WWAV strain AV 9310135, and other Tacaribe serocomplex viruses. Each sequence included a 43- to 74-nt fragment of the 5' NCR, the complete GP-C gene, intergenic region, and N protein gene, and a 29- to 38-nt fragment of the 3' NCR. Total RNA was isolated from monolayers of arenavirus-infected Vero E6 cells, using TRIzol® Reagent (Invitrogen Life Technologies, Inc., Carlsbad, CA). First-strand cDNA was synthesized by using SuperScript II RNase H⁻ Reverse Transcriptase (Invitrogen Life Technologies, Inc.) in conjunction with oligonucleotide 19C-cons (5'-CGCACMGWGGATCCTAGGC-3') (Cajimat et al., 2007). Amplicons were generated from 3 overlapping fragments of the arenavirus-specific first-strand cDNA by using the Master Taq Kit (Eppendorf North America, Inc., Westbury, NY) in conjunction with 19C-cons and AVGPC14 (5'-GGACAGCCYTCRCCRATKATGTGTCTGTG-3'), either AVGPC45 (5'-GAGTAARGARTATGAAGAGAGGC-3') and AVNP73 (5'-TAAGTGCAGAGCAGTCGCG-3'), AVGPC53 (5'-TGAGTGGATCTTGGAGAGTG-3') and AVNP74 (5'-GACATGCATGGTAGACAAGATC-3'), AVGPC53 and AVNP72 (5'-GTTGATGTGAAGCTAAGTGC-3'), or AVGPC90 (5'-GAAATGATTGGCTTGTGAAAGTG-3') and AVNP97 (5'-GATATGCATGGWAGACARGATTT-3'), and then AVNP13 (5'-GTTGKTCWGGYTCYCTGAA-3') and 19C-cons. Oligonucleotides 19C-cons and AVGPC14 flanked a 1,366- to 1,400-nt fragment that extended from within the 5' NCR into

the GP-C gene, AVGPC45 and AVNP73 flanked a 523-nt fragment of the S segment of AV D1000090 that extended from within the GPC gene through the stop codon of the N protein gene, AVGPC53 and AVNP74 flanked a 615-nt fragment of the S segment of AV D1240007 that extended from within the GPC gene through the stop codon of the N protein gene, AVGPC53 and AVNP72 flanked a 583-nt fragment of the S segment of AV 96010024 that extended from within the GPC gene through the stop codon of the N protein gene, AVGPC90 and AVNP97 flanked a 609-nt fragment of the S segment of AV 96010151 that extended from within the GPC gene through the stop codon of the N protein gene, and AVNP13 and 19C-cons flanked a 1,685- to 1,694-nt fragment that extended from within the N protein gene, through the start codon of the N protein gene, and into the 3' NCR. Both strands of each amplicon were sequenced directly, using the dye termination cycle sequencing technique (Applied Biosystems, Inc., Foster City, CA). The nucleotide sequences of the S segments of AV 96010024, AV 96010151, AV D1000090, and AV D1240007 were deposited into the GenBank nucleotide sequence database under Accession Nos. EU123331, EU123330, EU123328, and EU123329, respectively.

The analyses of the nucleotide and amino acid sequence data included BCNV strains AV A0060209 and AV 98470029 (GenBank Accession Nos. AF512833 and AY924392, respectively), CTNV strains AV A0400135 and AV A0400212 (DQ865244 and DQ865245, respectively), TAMV strain W 10777 (AF512828), WWAV strain AV 9310135 (AF228063), ALLV strain CLHP-2472 (AY012687), AMAV strain BeAn 70563 (AF512834), CPXV strain BeAn 119303 (AF512832), FLEV strain BeAn 293022 (AF512831), GTOV strain INH-95551 (NC_005077), JUNV strains XJ13, MC2, and Romero (NC_005081, D10072, and AY619641, respectively), LATV strain MARU 10924 (AF485259), MACV strains Carvalho, Chicava, Mallele, and 9530537 (NC_005078, AY624355, AY619645, and AY571959, respectively), OLVV strain 3229-1 (U34248), PARV strain 12056 (AF485261), PICV strain An 3739 (NC_006447), PIRV strain VAV-488 (NC_005894), SABV strain SPH 114202 (NC_006317), TCRV strain TRVL 11573 (NC_004293), and LCMV strain WE (M22138). Multiple strains of BCNV, CTNV, JUNV, and MACV were included in the analyses to provide a metric to assess the taxonomical significance of sequence nonidentities between the arenaviruses isolated from the Mexican woodrats and between the arenaviruses from the Mexican woodrats and other North American arenaviruses. The LCMV strain WE was included in the phylogenetic analyses to enable inference of the ancestral node within the group of New World arenaviruses.

The alignments of the predicted amino acid sequences of the GP-C and N proteins were generated by using the computer program CLUSTAL W1.7 (Thompson et al., 1994). The alignments of the nucleotide sequences of the GP-C genes and nucleotide sequences of the N protein genes were constructed manually based on the computer-generated alignments of the GP-C amino acid sequences and N protein amino acid sequences, respectively. The analyses of the nucleotide sequence alignments were done by using MRBAYES 3.1.2 (Huelsenbeck and Ronquist, 2001) and other programs in the computer software package PAUP, version 4.0b10 (Swofford, 2002). Sequence nonidentities were equivalent to uncorrected distances. A GTR+I+G model with a site-specific gamma distribution and sites partitioned by codon was used with the following options in MRBAYES 3.1.2: 4 Markov-chains, 1,000,000 generations, and sample frequency = every 1,000th generation. The first 1,000 trees were discarded after review of the likelihood scores, convergence statistics, and potential scale reduction factors. A consensus tree (50% majority rule) was constructed from the remaining trees and then clade probability values were generated to assess support for the nodes within the consensus tree.

3. Results

Arenavirus was isolated from the samples of spleen, kidney, and urine from TK119202, samples of spleen or kidney from 3 of 31 other Mexican woodrats captured near Skinner Tank, and samples of spleen, kidney, and urine from TK123380. None of the 4 virus-positive animals captured near Skinner Tank were antibody-positive to WWAV strain AV 9310135 (C. F. Fulhorst, unpublished data).

The lengths of the GP-C genes of AV 96010024, AV 96010151, AV D1000090, AV D1240007, WWAV strain AV 9310135, and the 5 other North American viruses ranged from 1,440- to 1,455-nt (Table 2). The lengths of the N protein genes of AV 96010024, AV 96010151, AV D1000090, AV D1240007, WWAV strain AV 9310135, and the 5 other North American viruses were identical (i.e., 1,686-nt).

Nonidentities between the nucleotide sequences of the GP-C genes and between the nucleotide sequences of the N protein genes of AV 96010024, AV 96010151, AV D1000090, AV D1240007, and WWAV strain AV 9310135 ranged from 21.5 to 33.7% and 14.5 to 24.9%, respectively (Table 3). Nonidentities between the nucleotide sequences of the GP-C genes and between the nucleotide sequences of the N protein genes of these viruses and the 5 other North American viruses ranged from 30.6 to 39.1% and 21.3 to 28.7%, respectively (Table 3).

The 2 trees generated by simultaneous Bayesian analyses of the GP-C gene nucleotide sequences were identical. Likewise, the 2 trees generated by simultaneous Bayesian analyses of the N protein gene nucleotide sequences were identical. The analyses of the GP-C gene sequences (Figure 2A) and the N protein gene sequences (Figure 2B) indicated that AV 96010024, AV 96010151, AV D1240007, and WWAV strain AV 9310135 are monophyletic and, as a group, phylogenetically distinct from AV D1000090 and the other North American viruses. Further, the analyses of the GP-C gene sequences indicated that the AV 96010024-AV 96010151-AV D1240007-WWAV strain AV 9310135 lineage is most closely related to the AV D1000090 lineage whereas the analyses of the N protein gene sequences indicated that the AV 96010024-AV 96010151-AV D1240007-WWAV strain AV 9310135 lineage is most closely related to the CTNV lineage. The clade probability values for monophyly of AV 96010024, AV 96010151, AV D1240007, and WWAV strain AV 9310135 in the analyses of the GP-C gene sequences and N protein gene sequences were 1.00, the clade probability values for monophyly of the AV 96010024-AV 96010151-AV D1240007-WWAV strain AV 9310135 lineage and AV D1000090 lineage in the analyses of the GP-C gene sequences were 1.00, and the clade probability values for monophyly of the AV 96010024-AV 96010151-AV D1240007-WWAV strain AV 9310135 lineage and the CTNV lineage in the analyses of the N protein gene sequences were 1.00. However, the clade probability values for monophyly of AV 96010151 and WWAV strain AV 9310135 in the analyses of the GP-C gene sequences were less than 0.95 and the clade probability value for monophyly of AV 96010024 and WWAV strain AV 9310135 in 1 of the analyses of the N protein sequences was less than 0.95. Thus, the phylogenetic relationships within the AV 96010024-AV 96010151-WWAV strain AV 9310135 lineage were not resolved by the analyses of the GP-C gene or N protein gene sequences.

Nonidentities between the amino acid sequences of the GP-C of strains of a single species ranged from 0.8% (JUNV strains XJ13 and Romero) to 5.6% (MACV strains Chicava and 9530537). Similarly, nonidentities between the amino acid sequences of the N proteins of strains of a single species ranged from 0.5% (BCNV strains AV A0060209 and AV 98470029) to 3.9% (JUNV strains XJ13 and MC2).

Nonidentities between the amino acid sequences of the GP-C and between the amino acid sequences of the N proteins of AV 96010024, AV 96010151, AV D1240007, and WWAV

strain AV 9310135 ranged from 16.0 to 25.8% and from 6.2 to 10.3%, respectively (Table 4). Thus, AV 96010024, AV 96010151, and AV D1240007 could be considered strains of WWAV or strains of novel arenavirus species that are phylogenetically closely related to WWAV.

Nonidentities between the amino acid sequences of the GP-C of strains of different South American arenavirus species ranged from 15.8 to 60.2%, nonidentities between the amino acid sequences of the GP-C of BCNV strain AV A0060209 or AV 98470029, CTNV strain AV A0400135 or AV A0400212, TAMV strain W 10777, and WWAV strain AV 9313105 ranged from 33.3 to 40.3% (Table 4), nonidentities between the amino acid sequence of the GP-C of AV D1000090 and the amino acid sequences of the GP-C of BCNV strains AV A0060209 and AV 98470029, CTNV strains AV A0400135 and AV A0400212, TAMV strain W 10777, and WWAV strain AV 9313105 ranged from 29.9 to 35.1% (Table 4), and nonidentities between the amino acid sequences of the GP-C of AV 96010024, AV 96010151, and AV D1240007 and the amino acid sequences of the GP-C of AV D1000090, WWAV strain AV 9310135, and the 5 other North American arenaviruses ranged from 19.2 to 41.1% (Table 4). Similarly, nonidentities between the amino acid sequences of the N proteins of strains of different South American arenavirus species ranged from 11.9 to 44.3%, nonidentities between the amino acid sequences of the N proteins of BCNV strain AV A0060209 or AV 98470029, CTNV strain AV A0400135 or AV A0400212, TAMV strain W 10777, and WWAV strain AV 9313105 ranged from 13.3 to 21.7% (Table 4), nonidentities between the amino acid sequence of the N protein of AV D1000090 and the amino acid sequences of the N proteins of BCNV strains AV A0060209 and AV 98470029, CTNV strains AV A0400135 and AV A0400212, TAMV strain W 10777, and WWAV strain AV 9313105 ranged from 10.9 to 19.0% (Table 4), and nonidentities between the amino acid sequences of the N proteins of AV 96010024, AV 96010151, and AV D1240007 and the amino acid sequences of the N proteins of AV D1000090, WWAV strain AV 9310135, and the 5 other North American arenaviruses ranged from 6.2 to 21.0% (Table 4). Thus, AV D1000090 is a strain of a novel arenavirus species. The name *Skinner Tank virus* (SKTV) is proposed to distinguish the species represented by AV D1000090 from WWAV and all other arenavirus species.

4. Discussion

The *Eighth Report of the International Committee on Taxonomy of Viruses* (Salvato et al., 2005) indicated that strains of different arenavirus species should exhibit significant differences in pairwise comparisons of amino acid sequences or significant differences in two-way serological tests. In this study, nonidentities between the amino acid sequences of the GP-C and between the amino acid sequences of the N proteins of strains of different South American arenavirus species were as low as 15.8% and 11.9%, respectively, and nonidentities between the amino acid sequences of the GP-C and between the amino acid sequences of the N proteins of AV 96010024, AV 96010151, AV D1240007, and WWAV strain AV 9310135 ranged from 16.0 to 25.8% and 6.2 to 10.3%, respectively (Table 4). Thus, AV 96010024, AV 96010151, and/or AV D1240007 could be considered strains of WWAV or strains of novel arenavirus species that are phylogenetically most closely related to WWAV. Alternatively, AV 96010024, AV 96010151, AV D1240007, and WWAV strain AV 9310135 could be grouped together in a *Whitewater Arroyo virus* species complex.

The rodent fauna of McKinley County, New Mexico includes the white-throated woodrat (*N. albigula*) and Mexican woodrat (*N. mexicana*) (Findley et al. 1975). Presently, our knowledge of the natural host relationships of WWAV is limited to the isolation of the prototype strain AV 9310135 and 2 other WWAV strains from 2 (12.5%) of 16 white-throated woodrats (*N. albigula*) captured near the town of Gallup in McKinley County (Fulhorst et al., 1996). Hypothetically, the WWAV infections in these white-throated woodrats were a consequence of interspecific (i.e., *N. mexicana*-to-*N. albigula*) virus transmission. If so, then membership

in the *Whitewater Arroyo virus* species complex could be restricted to arenaviruses that are principally associated with the Mexican woodrat (*N. mexicana*).

Humans usually become infected with arenaviruses following inhalation of infectious virus in aerosolized droplets of respiratory secretions, other secretions, or urine from rodents. The isolation of arenavirus from the samples of urine from TK119202 and TK123380 indicate that urine from infected Mexican woodrats may be infectious to humans.

Six arenaviruses naturally cause severe febrile disease in humans: LCMV, LASV, GTOV, JUNV, MACV, and SABV (Peters, 2002). The human health significance of arenaviruses associated with woodrats and other cricetid rodents indigenous to the western United States is the subject of research supported by the United States Public Health Service, National Institutes of Health.

The analyses of the nucleotide sequences of the GP-C genes and N protein genes in this study revealed a high level of genetic diversity among arenaviruses naturally associated with Mexican woodrats and other cricetid rodents native to the western United States. Knowledge of the nucleotide sequences of the GP-C genes and N proteins genes of the North American arenaviruses in this study may prove useful in future efforts to develop accurate assays for arenavirus-specific RNA in acute-phase biological specimens from fever patients who live or work in close association with cricetid rodents native to the western United States.

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Figure 1.

Map of the study area showing the locations at which the arenavirus-positive Mexican woodrats (*Neotoma mexicana*) were captured: 1, AV 96010024 (San Juan County, Utah); 2, *Skinner Tank virus* strain AV D1000090 (Coconino County, Arizona); 3, AV D1240007 (Larimer County, Colorado); 4, AV 96010151 (Socorro County, New Mexico).

Figure 2.

Phylogenetic relationships among 29 New World arenaviruses based on Bayesian analyses of (2A) glycoprotein precursor gene sequences and (2B) nucleocapsid protein gene sequences. The scale bar indicates a sequence divergence equivalent to 100 changes. The number(s) at the nodes are clade probability values. A single “1.00” indicates that the clade probability values for both analyses were 1.00. Clade probability values less than 0.93 were not included in the phylograms. The branch labels in 2B include (in the following order) viral species, strain, host species, and country. ALLV, *Allpahuayo virus*; AMAV, *Amapari virus*; BCNV, *Bear Canyon virus*; CPXV, *Cupixi virus*; CTNV, *Catarina virus*; FLEV, *Flexal virus*; GTOV, *Guanarito virus*; JUNV, *Junín virus*; LATV, *Latino virus*; LCMV, *lymphocytic choriomeningitis virus*; MACV, *Machupo virus*; OLVV, *Oliveros virus*; PARV, *Paraná virus*; PICV, *Pichindé virus*; PIRV, *Pirital virus*; SABV, *Sabiá virus*; SKTV, *Skinner Tank virus*; TCRV, *Tacaribe virus*; TAMV, *Tamiami virus*; WWAV, *Whitewater Arroyo virus*. Arg, Argentina; Bol, Bolivia; Bra, Brazil; Col, Colombia; Par, Paraguay; Per, Peru; Tri, Trinidad; USA, United States of America (Az, Arizona; Ca, California; Co, Colorado; Fl, Florida; NM, New Mexico; Tx, Texas; Ut, Utah); Ven, Venezuela. Aspp, *Artibeus* species (frugivorous bats); Bobs, *Bolomys obscurus* (dark bolo mouse); Ccal, *Calomys callosus* (large vesper mouse); Csp, *Calomys* species; Hsap, *Homo sapiens* (human); Nalb, *Neotoma albigula* (white-throated woodrat); Ngui, *Neacomys guianae* (Guiana bristly mouse); Nmac, *Neotoma macrotis* (large-eared woodrat); Nmex, *Neotoma mexicana* (Mexican woodrat); Nmic, *Neotoma micropus* (southern plains woodrat); Oalb, *Oryzomys albigularis* (Tomes’s oryzomys); Obic, *Oecomys bicolor* (bicolored arboreal rice rat); Obuc, *Oryzomys buccinatus* (Paraguayan rice rat); Ocap, *Oryzomys capito* (large-headed rice rat); Osp, *Oryzomys* species; Pcal, *Peromyscus californicus* (California mouse); Sals, *Sigmodon alstoni* (Alston’s cotton rat); Shis, *Sigmodon hispidus* (hispid cotton rat). The LCMV strain WE is an Old World arenavirus and was included in the analyses to enable inference of the ancestral node among the New World arenaviruses.

Table 1
Arenaviruses isolated from Mexican woodrats (*Neotoma mexicana*) captured in the western United States

Strain	Museum number	Month-year captured	State	County	Locality
AV 96010024	NK36282	Jul 1994	Utah	San Juan	Natural Bridges National Monument
AV 96010151	NK62425	Sep 1994	New Mexico	Socorro	Magdalena Mountains
AV D1000090	TK119202	Aug 2002	Arizona	Coconino	Skinner Tank
AV D1240007	TK123380	Oct 2002	Colorado	Larimer	Big Thompson Canyon

Table 2

Lengths of the glycoprotein precursor genes and nucleocapsid protein genes of 10 North American arenaviruses

Species ^a	Strain	Length (nt) ^b	
		GP-C	N protein
SKTV	AV D1000090	1443	1686
--	AV D1240007	1452	1686
--	AV 96010024	1440	1686
--	AV 96010151	1449	1686
BCNV	AV A0060209	1449	1686
BCNV	AV 98470029	1449	1686
CTNV	AV A0400135	1452	1686
CTNV	AV A0400212	1452	1686
TAMV	W 10777	1455	1686
WWAV	AV 9310135	1440	1686

^aBCNV, *Bear Canyon virus*; CTNV, *Catarina virus*; SKTV, *Skinner Tank virus*; TAMV, *Tamiami virus*; WWAV, *Whitewater Arroyo virus*.

^bGP-C, glycoprotein precursor gene; N protein, nucleocapsid protein gene.

Nonidentities between the nucleotide sequences of the glycoprotein precursor genes and between the nucleotide sequences of the nucleocapsid protein genes of 10 North American arenaviruses^a

Table 3

Species ^b	Strain ^c	Glycoprotein precursor gene (% sequence nonidentity)									
		AV 96010024	AV 96010151	AV D124007	AV A0060209	AV 98470029	AV A0400135	AV A0400212	AV D1000090	W 10777	AV 9310135
--	AV 96010024	--	21.5	28.1	37.1	37.3	34.2	33.3	31.9	36.2	23.4
--	AV 96010151	15.5	---	28.5	36.5	36.6	34.3	32.1	31.6	36.8	21.6
--	AV D124007	17.2	17.3	--	37.4	37.5	34.5	34.2	33.3	39.1	28.8
BCNV	AV A0060209	28.1	27.9	27.9	--	3.0	35.9	34.7	37.4	34.9	37.8
BCNV	AV 98470029	28.7	28.0	28.0	3.6	--	35.5	34.4	37.5	34.7	37.8
CTNV	AV A0400135	23.0	22.4	21.3	27.8	28.4	--	9.4	30.6	36.7	34.7
CTNV	AV A0400212	23.4	21.8	22.1	27.5	27.3	9.6	--	30.7	36.6	33.7
SKTV	AV D1000090	31.9	31.6	33.3	37.4	37.5	30.6	30.7	--	35.4	33.7
TAMV	W 10777	27.5	27.3	27.7	28.2	28.2	27.0	27.8	35.4	--	37.3
WWAV	AV 9310135	16.0	14.5	19.2	27.7	28.1	22.9	22.1	33.7	28.3	--

Nucleocapsid protein gene (% sequence nonidentity)

^aNumbers above and below the diagonal line are the nonidentities between the nucleotide sequences of the glycoprotein precursor genes and between the nucleotide sequences of the nucleocapsid protein genes, respectively.

^bBCNV = Bear Canyon virus; CTNV = Skinner Tank virus; TAMV, *Tamiami virus*; WWAV, *Whitewater Arroyo virus*.

^cStrains AV 96010024, AV 96010151, AV D124007, and AV D1000090 were isolated from Mexican woodrats (*Neotoma mexicana*) captured in Utah, New Mexico, Colorado, and Arizona, respectively; AV A0060209 was isolated from a California mouse (*Peromyscus californicus*) captured in Orange County, California; AV 98470029 was isolated from a large-eared woodrat (*Neotoma macrotis*) captured in Riverside County, California; AV A0400135 and AV A0400212 were isolated from southern plains woodrats (*Neotoma micropus*) captured in Dimmit and La Salle Counties, Texas, respectively; W 10777 was isolated from a hispid cotton rat (*Sigmodon hispidus*) captured in Monroe County, Florida; AV 9310135 was isolated from a white-throated woodrat (*Neotoma albigula*) captured in McKinley County, New Mexico.

Table 4

Nonidentities between the predicted amino acid sequences of the glycoprotein precursors and between the predicted amino acid sequences of the nucleocapsid proteins of 10 North American arenaviruses^a

Species ^b	Strain ^c	Glycoprotein precursor (% amino acid sequence nonidentity)									
		AV 96010024	AV 96010151	AV D1240007	AV A0060209	AV 98470029	AV A0400135	AV A0400212	AV D1000090	W 10777	AV 9310135
--	AV 96010024	--	16.0	25.8	38.2	38.4	33.3	33.5	31.0	38.3	19.8
--	AV 96010151	7.3	--	25.5	38.8	39.0	32.7	33.3	30.1	38.3	19.2
--	AV D1240007	10.3	7.3	--	39.7	39.9	34.1	35.5	32.0	41.1	25.6
BCNV	AV A0060209	21.0	19.2	19.9	--	1.7	35.6	35.6	35.1	34.7	40.0
BCNV	AV 98470029	20.6	18.9	19.6	0.5	--	35.1	35.3	34.9	34.3	40.3
CTNV	AV A0400135	14.6	12.6	13.2	18.1	18.0	--	4.8	29.9	35.7	33.3
CTNV	AV A0400212	14.1	12.3	13.0	17.8	17.4	1.6	--	30.6	36.1	33.8
SKTV	AV D1000090	15.3	13.9	15.5	17.4	17.1	11.4	10.9	--	33.6	33.3
TAMV	W 10777	20.8	19.2	19.4	21.7	21.5	18.5	18.1	19.0	--	38.7
WWAV	AV 9310135	9.3	6.2	9.1	18.7	18.3	13.5	13.3	14.2	19.9	--
Nucleocapsid protein (% amino acid sequence nonidentity)											

^aNumbers above and below the diagonal line are the nonidentities between the predicted amino acid sequences of the glycoprotein precursors and between the predicted amino acid sequences of the nucleocapsid proteins, respectively.

^bBCNV, Bear Canyon virus; CTNV, Skinner Tank virus; TAMV, Tamiami virus; WWAV, Whitewater Arroyo virus.

^cStrains AV 96010024, AV 96010151, AV D1240007, and AV D1000090 were isolated from Mexican woodrats (*Neotoma mexicana*) captured in Utah, New Mexico, Colorado, and Arizona, respectively; AV A0060209 was isolated from a California mouse (*Peromyscus californicus*) captured in Orange County, California; AV 98470029 was isolated from a large-eared woodrat (*Neotoma macrotis*) captured in Riverside County, California; AV A0400135 and AV A0400212 were isolated from southern plains woodrats (*Neotoma micropus*) captured in Dimmit and La Salle Counties, Texas, respectively; W 10777 was isolated from a hispid cotton rat (*Sigmodon hispidus*) captured in Monroe County, Florida; AV 9310135 was isolated from a white-throated woodrat (*Neotoma albigula*) captured in McKinley County, New Mexico.