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Principal host relationships and evolutionary history of the North American arenaviruses

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Abstract

A previous study suggested that the genomes of the arenaviruses native to North America are a product of genetic recombination between New World arenaviruses with significantly different phylogenetic histories. The purpose of this study was to extend our knowledge of the principal host relationships and evolutionary history of the North American arenaviruses. The results of this study suggest that the large-eared woodrat (*Neotoma macrotis*) is a principal host of Bear Canyon virus and that the present-day association of Bear Canyon virus with the California mouse (*Peromyscus californicus*) in southern California represents a successful host-jumping event from the large-eared woodrat to the California mouse. Together, the results of analyses of viral gene sequence data in this study and our knowledge of the phylogeography of the rodents that serve as principal hosts of the New World arenaviruses suggest that genetic recombination between arenaviruses with significantly different phylogenetic histories did not play a role in the evolution of the North American arenaviruses.

Keywords

Arenaviridae; Bear Canyon virus; Tamiami virus; Whitewater Arroyo virus; Z protein; RNA-dependent RNA polymerase

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Introduction

The virus family *Arenaviridae* comprises 2 serocomplexes and 22 species (Salvato et al., 2005). The Tacaribe (New World) complex 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), *Junin virus* (JUNV), *Latino virus* (LATV), *Machupo virus* (MACV), *Oliveros virus* (OLVV), *Parana virus* (PARV), *Pichindé virus* (PICV), *Pirital virus* (PIRV), and *Sabiá virus* (SABV) in South America. The lymphocytic choriomeningitis-Lassa (Old World) complex includes *Ippy virus* (IPPYV), *Lassa virus* (LASV), *lymphocytic choriomeningitis virus* (LCMV), *Mobala virus* (MOBV), and *Mopeia virus* (MOPV).

Specific members of the subfamilies Neotominae and Sigmodontinae in the rodent family Cricetidae (Wilson and Reeder, 2005) are the principal hosts of the New World arenaviruses for which natural host relationships have been well characterized. For example, the hispid cotton rat (*Sigmodon hispidus*) in southern Florida is the principal host of TAMV (Calisher et al., 1970; Jennings et al., 1970), the white-throated woodrat (*Neotoma albigula*) in northwestern New Mexico is the principal host of WWAV (Fulhorst et al., 1996), and Alston's cotton rat (*Sigmodon alstoni*) in western Venezuela is the principal host of PIRV (Fulhorst et al., 1997, 1999).

The California mouse (*Peromyscus californicus*) is a natural host and may be a principal host of BCNV (Fulhorst et al., 2002). Bear Canyon is located in the Santa Ana Mountains in western Riverside County near the Riverside County-Orange County line. The BCNV prototype strain AV A0070039 was isolated from a California mouse captured in November 1998 in Riverside County near Bear Canyon (Fulhorst et al., 2002). Strains of BCNV subsequently were isolated from 4 (19.1%) of 21 California mice captured in June 1998 at 2 sites in the Santa Ana Mountains in eastern Orange County (Fulhorst et al., 2002).

In previous studies (Bennett et al., 2000; Fulhorst et al., 2002), antibody to an arenavirus was found in 1 (50.0%) of 2 dusky-footed woodrats (*Neotoma fuscipes*) and 4 (50.0%) of 8 California mice captured in western Riverside County near Bear Canyon, 5 (16.1%) of 31 dusky-footed woodrats and 7 (21.9%) of 32 California mice captured at 7 sites in eastern Orange County, and 3 (6.8%) of 44 dusky-footed woodrats and 1 (4.2 %) of 24 California mice captured at 14 sites in southern Orange County. Note that the large-eared woodrat (*Neotoma macrotis*) in Riverside County, Orange County, Los Angeles County, and other counties in southern California recently was elevated from subspecific status within the *N. fuscipes* species complex (Matocq, 2002). Thus, the antibody-positive woodrats captured in Riverside County and in Orange County in the previous studies likely were large-eared woodrats, not dusky-footed woodrats.

Studies done in the 1990′s established that multiple (different) arenaviruses coexist in certain regions of South America. For example, JUNV and OLVV are sympatric in the pampas of northern Argentina (Mills et al., 1996) and GTOV and PIRV are sympatric on the plains of western Venezuela (Fulhorst et al., 1997, 1999). Hypothetically, BCNV is not the arenavirus associated with the large-eared woodrat in the Santa Ana Mountains. The first objective of the present study was to determine the identity of the arenavirus associated with the large-eared woodrat in southern California.

Arenaviruses possess genomes that consist of 2 single-stranded RNA segments, designated small (S) and large (L) (Southern, 1996). The S segment (~3.5 kb) consists of a 5′ non-coding region (NCR), the glycoprotein precursor (GPC) gene, an intergenic region that separates the

GPC gene from the nucleocapsid (N) protein gene, the N protein gene, and a 3′ NCR. Similarly, the L segment (~7.3 kb) consists of a 5′ 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.

Independent analyses of complete GPC sequences and complete N protein sequences in a previous study (Charrel et al., 2002) delineated 5 phylogenetic lineages within the *Arenaviridae*: North American (BCNV, TAMV, and WWAV), South American lineage A (ALLV, FLEV, PARV, PICV, and PIRV), South American lineage B (AMAV, CPXV, GTOV, JUNV, MACV, SABV, and TCRV), South American lineage C (LATV and OLVV), and Old World (LASV, LCMV and MOPV). Note that the analysis of the GPC sequence data placed the North American lineage in a sister relationship to the South American lineage B whereas the analysis of the N protein sequence data placed the North American lineage in a sister relationship to the South American lineage A. Also note that the monophyly of the North American lineage and the South American lineage B in the analysis of the GPC sequence data and the monophyly of the North American lineage and the South American lineage A in the analysis of the N protein sequence data were strongly supported by the results of bootstrap analyses (Felsentein, 1985).

Previously, our most comprehensive knowledge of the evolutionary history of the arenaviral L genomic segment was based on analyses of amino acid sequences predicted from a small fragment (288- to 300-nt) of the RdRp genes of BCNV strain AV A0060209, TAMV strain W-10777, WWAV strain AV 9310135, TCRV strain TRVL II573, 11 South American arenaviruses, and 3 Old World arenaviruses (Charrel et al., 2003). In a neighbor-joining analysis of the RdRp sequence data, the 3 North American arenaviruses were monophyletic, the North American lineage was sister to a lineage that comprised ALLV, PICV and PIRV, and the BCNV-TAMV-WWAV-ALLV-PICV-PIRV lineage was sister to a lineage that comprised AMAV, CPXV, GTOV, JUNV, MACV, SABV and TCRV. Thus, the L segments (RdRp genes) and the 3′ halves of the S segments (N protein genes) of the North American arenaviruses appear to be descended from an ancestor of the South American lineage A viruses that emerged after the divergence of the South American lineage A from the South American lineage B. The second objective of this study was to extend and refine our knowledge of the phylogenetic history of the North American arenaviruses, specifically -- to determine whether the L genomic segments of BCNV, TAMV, and WWAV are a product of homologous recombination between the L segment of a South American lineage A virus and the L segment of a South American lineage B virus.

Results

Strain AV 98470029 is an arenavirus that was isolated in this study from a large-eared woodrat captured in September 1998 in the Santa Ana Mountains in Riverside County. The BCNV strains AV A0060209 and AV A0070039 were isolated in a previous study (Fulhorst et al., 2002) from California mice captured in the Santa Ana Mountains in Orange County and Riverside County, respectively. The nucleotide sequences of the GPC and N protein genes of AV 98470029 were compared to the homologous sequences of AV A0060209, AV A0070039, TAMV strain W-10777, WWAV strain AV 9310135, TCRV strain TRVL II573, and 7 South American arenaviruses (Table 1). In pairwise comparisons, the nucleotide sequence of the GPC gene of AV 98470029 was 97.0%, 98.1%, 64.0%, and 62.5% identical to the nucleotide sequence of the GPC gene of BCNV strain AV A0060209, BCNV strain AV A0070039, TAMV strain W-10777, and WWAV strain AV 9310135, respectively, and less than 57.3% identical to the nucleotide sequences of the GPC genes of TCRV strain TRVL II573 and the 7 South American arenaviruses included in this study (Table 2). Similarly, the nucleotide sequence of the N protein gene of AV 98470029 was 96.4%, 98.0%, 71.8%, and 71.9%

identical to the nucleotide sequence of the N protein gene of BCNV strain AV A0060209, BCNV strain AV A0070039, TAMV strain W-10777, and WWAV strain AV 9310135, respectively, and less than 63.9% identical to the nucleotide sequences of the N protein genes of TCRV strain TRVL II573 and the 7 South American arenaviruses included in this study (Table 2). Neighbor-joining analyses of the *p* model distances generated from the GPC and N protein gene sequence alignments (all 3 nucleotide positions included in the calculation of the genetic distances) indicated that AV 98470029 is phylogenetically more closely related to BCNV strains AV A0070039 and AV A0060209 than to TAMV strain W-10777 or WWAV strain AV 9310135 (Figure 1). Together, the high level of sequence identity between strain AV 98470029 and strains AV A0060209 and AV A0070039 and the results of the neighbor-joining analyses of the nucleotide sequence data indicate that AV 98470029 is a strain of BCNV.

The Z genes of BCNV strain AV A0070039, TAMV strain W-10777, and WWAV strain AV 9310135 were similar in length to the Z genes of the 8 other New World arenaviruses included in this study, LASV strain Josiah, and LCMV strain WE (Appendix 1). In pairwise comparisons of the Z gene sequences, nucleotide sequence identities between the North American viruses ranged from 68.1% to 75.1%, sequence identities between the North American viruses and PICV strain An 3739 and PIRV strain VAV-488 ranged from 60.4% to 62.8%, and sequence identities between the North American arenaviruses and the 6 other New World arenaviruses included in the Z gene sequence alignment ranged from 44.9% to 53.0% (Table 3). The neighbor-joining analysis of the *p* model distances generated from the Z gene sequence alignment (all 3 nucleotide positions included in the calculation of the genetic distances) indicated that the North American arenaviruses are monophyletic and phylogenetically more closely related to PICV and PIRV than to the 6 other New World arenaviruses included in the Z gene sequence alignment (Figure 2).

The RdRp genes of BCNV strain AV A0070039, TAMV strain W-10777, and WWAV strain AV 9310135 were similar in length to the RdRp genes of the 8 other New World arenaviruses included in this study, LASV strain Josiah, and LCMV strain WE (Appendix 1). In pairwise comparisons of the RdRp gene sequences, nucleotide sequence identities between the North American viruses ranged from 66.5% to 67.9%, sequence identities between the North American viruses and PICV strain An 3739 and PIRV strain VAV-488 ranged from 56.5% to 57.8%, and sequence identities between the North American viruses and the 6 other New World arenaviruses included in the RdRp gene sequence alignment ranged from 49.7% to 51.4% (Table 3). The neighbor-joining analysis of the *p* model distances generated from the RdRp gene sequence alignment (all 3 nucleotide positions included in the calculation of the genetic distances) indicated that the North American arenaviruses are monophyletic and phylogenetically more closely related to PICV and PIRV than to the 6 other New World arenaviruses included in the RdRp gene sequence alignment (Figure 2).

Discussion

The results of a previous study (Bowen et al., 1997) indicated that the principal host relationships of some New World arenaviruses represent a long-term shared evolutionary relationship between the *Arenaviridae* and the Sigmodontinae. The evidence for this long-standing relationship includes the present-day association of phylogenetically closely related arenaviruses with phylogenetically closely related sigmodontine rodents, for example -- JUNV with the drylands vesper mouse (*Calomys musculinus*) in Argentina and MACV with the large vesper mouse (*Calomys callosus*) in Bolivia (Childs and Peters, 1993). The present-day principal host relationships of some of the other sigmodontine rodent-associated arenaviruses may be a consequence of “host-jumping” or “host-switching” events that involved different sigmodontine rodents or neotomine rodents and sigmodontine rodents.

The isolation of strain AV 98470029 from a large-eared woodrat in this study is the first direct evidence that BCNV is naturally associated with a rodent other than the California mouse. Other direct evidence that BCNV is naturally associated with the large-eared woodrat includes the isolation of BCNV strain AV B0300052 (GenBank accession no. EF089388) from a large-eared woodrat captured in Zuma Canyon in the Santa Monica Mountains in Los Angeles County, California (M. P. Rood, personal communication). Zuma Canyon is located approximately 140 km northwest of Bear Canyon. The isolation of BCNV strains AV 98470029 and AV B0300052 from large-eared woodrats captured at different sites in southern California suggest that the large-eared woodrat is a principal host of BCNV.

The hallmark of the arenaviruses is their ability to establish chronic infections in their principal hosts. It generally is assumed that the long-term persistence of an arenavirus in nature is dependent upon the capacity of chronically infected rodents to transmit their infections to subsequent generations of conspecifics. Accordingly, future research on the ecology of BCNV should include studies on the duration of BCNV infection in naturally infected large-eared woodrats, studies on the duration of BCNV infection in naturally infected California mice, and studies on the ability of naturally infected large-eared woodrats and California mice to initiate intraspecific virus transmission.

Collectively, the results of the neighbor-joining analyses of the Z, RdRp, and N protein gene sequence data in this study support the notion that the L segments and the 3′ halves of the S segments of the North American arenaviruses are descended from an ancestor of the South American lineage A viruses that emerged after the last common ancestor of the South American lineage A and South American lineage B viruses. In contrast, the results of the neighbor-joining analysis of the GPC gene sequence data in this study, as in previous studies (Archer and Rico-Hesse, 2002; Charrel et al., 2001, 2002), suggest that the 5′ halves of the S segments of the North American arenaviruses are descended from an ancestor of the South American lineage B viruses that emerged after the last common ancestor of the South American lineage A and South American lineage B viruses. The difference in the placement of the North American lineage with respect to the South American lineages was attributed in previous studies (Archer and Rico-Hesse, 2002; Charrel et al., 2001, 2002) to homologous recombination between the S segment of a lineage A virus and the S segment of a lineage B virus. An alternative explanation for the difference in the placement of the North American lineage with respect to the South American lineages is that the evolution of the GPC genes of the North American arenaviruses has been affected by selection pressures more similar to the selection pressures that shaped the evolution of the GPC genes of the South American lineage B viruses than to the selection pressures that shaped the evolution of the GPC genes of the South American lineage A viruses. Note that this alternative explanation does not entail genetic recombination between a South American lineage A virus and a South American lineage B virus.

The known geographical range of the arenaviruses native to the New World extends from southern California (Fulhorst et al., 2002), southern Utah (Fulhorst et al., 2001), and southern Florida (Calisher et al., 1970) southward to eastern Bolivia and southern Brazil (Childs and Peters, 1993). The Neotominae is exclusively North American and includes the California mouse, the white-throated woodrat, and other woodrats (*Neotoma* species) that are naturally associated with arenaviruses native to North America (Fulhorst et al., 2001). The Sigmodontinae includes the hispid cotton rat (*S. hispidus*) and 11 rodents (species) that serve as principal hosts of arenaviruses native to South America. These rodents are the bicolored arboreal oryzomys (*Oecomys bicolor*) in Peru (ALLV), Guiana bristly mouse (*Neacomys guianae*) in Brazil (AMAV), large-headed oryzomys (*Oryzomys capito*) in Brazil (CPXV), an oryzomys (*Oryzomys* sp.) in Brazil (FLEV), short-tailed cane mouse (*Zygodontomys brevicauda*) in Venezuela (GTOV), drylands vesper mouse (*C. musculinus*) in Argentina (JUNV), large vesper mouse (*C. callosus*) in Bolivia (LATV and MACV), dark bolo mouse

(*Bolomys obscurus*) in Argentina (OLVV), Paraguayan oryzomys (*Oryzomys buccinatus*) in Paraguay (PARV), Tomes's oryzomys (*Oryzomys albigularis*) in Colombia (PICV), and Alston's cotton rat (*S. alstoni*) in Venezuela (PIRV). Note that *Sigmodon* is the only genus that includes the principal host of a North American arenavirus (TAMV) and the principal host of a South American lineage A virus (PIRV).

The present-day geographical range of the genus *Neotoma* extends from British Columbia southward to Nicaragua and from peninsular Florida westward to coastal California (Wilson and Reeder, 2005). In a previous study (Fulhorst et al., 2001), arenaviruses phylogenetically closely related to WWAV were isolated from white-throated woodrats (*N. albigula*) captured in northwestern New Mexico and western Oklahoma, a bushy-tailed woodrat (*Neotoma cinerea*) captured in southern Utah, Mexican woodrats (*Neotoma mexicana*) captured in central New Mexico and southern Utah, and southern plains woodrats (*Neotoma micropus*) captured in southern Texas. *Neotoma albigula*, *N. cinerea*, *N. mexicana*, and *N. micropus* represent 3 of the 4 major phylogenetic subdivisions in the genus *Neotoma* (Edwards and Bradley, 2002). The broad geographical distribution of WWAV and WWA-like viruses in association with distantly related *Neotoma* species indicates that the present-day association between the North American lineage in the *Arenaviridae* and the genus *Neotoma* was established long ago.

Collectively, the "ancient" relationship between the *Arenaviridae* and the genus *Neotoma*, the close phylogenetic relationship between BCNV and WWAV (and between BCNV and other arenaviruses naturally associated with woodrats in the western United States), and the isolation of BCNV from large-eared woodrats captured in different counties in southern California suggest that the association of BCNV with the large-eared woodrat was established long ago. Hypothetically, the association of BCNV with the California mouse in southern California represents a successful "host-jumping" event from the large-eared woodrat to the California mouse.

The divergence of the sigmodontine rodents from the neotomine rodents has been dated to 18.1 to 16.8 million years ago (Steppan et al., 1994). The available fossil record suggests that sigmodontine rodents originally invaded South America from North America 3.5 to 2.5 million years ago. Thus, the South American arenaviruses likely are descended from an arenavirus (or arenaviruses) that originated in North America.

As a group, the association of PIRV with Alston's cotton rat in western Venezuela, ALLV with the bicolored arboreal oryzomys in Peru, FLEV with an oryzomyine rodent in Brazil, PICV with Tomes's oryzomys in Colombia, and PARV with the Paraguayan oryzomys in Paraguay suggest that the relationship between the South American lineage A in the *Arenaviridae* and the Sigmodontinae was established long ago -- maybe as early as the divergence of the sigmodontine rodents from the neotomine rodents. Assuming that the association of PIRV with Alston's cotton rat represents a long-term shared evolutionary relationship between the South American lineage A and the Sigmodontinae, then the present-day association of TAMV with *S. hispidus* in southern Florida likely was established after the hispid cotton rat diverged from Alston's cotton rat. Perhaps the present-day association of TAMV with the hispid cotton rat represents a successful host-jumping event from a neotomine rodent such as the eastern woodrat (*Neotoma floridana*) to the hispid cotton rat.

Conceptually, a successful host-jumping event could lead to an increase in the variety of habitats occupied by infected rodents if the habitat preferences of the new principal host are not the same as the habitat preferences of the original principal host. A successful host-jumping event also could lead to an expansion of the geographical range of infected rodents if the new principal host occurs outside the geographical region occupied by the original principal host.

As such, a successful host-jumping event may result in a change in the epidemiology of human disease caused by a particular arenavirus.

Materials and Methods

The nucleotide sequence of a 3290-nt fragment of the S segment of BCNV strain AV 98470029, a 3301-nt fragment of the S segment and a 7064-nt fragment of the L segment of the BCNV prototype strain AV A0070039 (Fulhorst et al., 2002), a 7103-nt fragment of the L segment of the TAMV prototype strain W-10777 (Calisher et al., 1970), a 7094-nt fragment of the L segment of the WWAV prototype strain AV 9310135 (Fulhorst et al., 1996) and a 7008-nt fragment of the L segment of AMAV strain BeAn 70563 (Pinheiro et al., 1966) were determined in this study. The S segment sequences each extend from within the 5′ NCR through the 3′ end of the N protein gene. Similarly, the L segment sequences each extend from within the 5′ NCR through the 3′ end of the RdRp gene. The sequence of the L segment of AMAV strain BeAn 70563 was determined to improve the representation of the South American arenaviruses in this study.

Virus assay

The spleen of a large-eared woodrat and the spleens of 6 cactus mice (*Peromyscus eremicus*) were tested for infectious arenavirus by cultivation in monolayers of Vero E6 cells as described previously (Fulhorst et al., 1996). The woodrat and the cactus mice were captured in September 1998 at a site in Riverside County located approximately 17.7 km southeast of Bear Canyon. Strain AV 98470029 was isolated from the spleen of the woodrat. The virus isolation attempts on the spleens of the 6 cactus mice were negative.

Preparation of RNA and synthesis of first-strand cDNA

Total RNA was isolated from monolayers of Vero E6 cells infected with strain AV 98470029, BCNV strain AV A0070039, TAMV strain W-10777, WWAV strain AV 9310135 or AMAV strain BeAn 70563, using TRIzol® Reagent (Invitrogen Life Technologies, Inc., Carlsbad, CA). Reverse transcription of arenavirus-specific RNA was carried out as described previously (Cajimat and Fulhorst, 2004), using SuperScript II RNase H$^-$ Reverse Transcriptase (Invitrogen Life Technologies, Inc.) in conjunction with 19C-cons (5′-CGCACMGWGGATCCTAGGC-3′). Oligonucleotide 19C-cons is a derivative of oligonucleotide ARE3′-END (Gonzalez et al., 1995) and was expected to anneal to the 19-nt fragment at the extreme 3′ end of the S segment and to the 19-nt fragment at the extreme 3′ end of the L segment (Auperin et al., 1982; Southern, 1996). The nucleotide sequence of the 19-nt fragment at the extreme 5′ end of the S segment and the nucleotide sequence of the 19-nt fragment at the extreme 5′ end of the L segment presumably are inverse complementary to the nucleotide sequence of the 19-nt fragment at the extreme 3′ end of the S segment and the nucleotide sequence of the 19-nt fragment at the extreme 3′ end of the L segment, respectively (Auperin et al., 1982; Djavani et al., 1997; Southern, 1996). Thus, 19C-cons was expected to prime synthesis of first-strand cDNA from 4 different templates: the 3′ end of the S segment, the 3′ end of the replicative intermediate synthesized from the S segment, the 3′ end of the L segment and the 3′ end of the replicative intermediate synthesized from the L segment.

PCR amplification of arenavirus-specific first-strand cDNA and sequencing reactions

The nucleotide sequence of the 3290-nt fragment of the S segment of strain AV 98470029 and the 3301-nt fragment of the S segment of strain AV A0070039 each were determined from 2 overlapping PCR products, S1 and S2. These PCR products were synthesized by using Hot Master *Taq* DNA polymerase (Eppendorf, Westbury, NY) in conjunction with 19C-cons *and* AVNP4 (Fulhorst et al., 2002) and 19C-cons *and* AVNP13 (5′-

GTTGTKTCWGGYTCYCTGAA-3′), respectively. Both strands of each PCR product were sequenced directly.

The nucleotide sequence of the 7064-nt fragment of the L segment of BCNV strain AV A0070039 was determined from 4 PCR products. BCNVL1 (5045-bp) and BCNVL2 (2196-bp) were generated from the AV A0070039 first-strand cDNA by using the Triple Master PCR System (Eppendorf) in conjunction with 19C-cons *and* AVPOL16 (5′-TCACTTATTAACAGAAGCCC-3′) and 19C-cons *and* AVPOL1 (5′-GAAYTTCTCAAAACATTTGATTTG-3′), respectively. The nucleotide sequence of 1 strand of each PCR product was determined directly. The BCNVL1-BCNVL2 sequence comprised the entire 7064-nt fragment of the L segment of AV A0070039. The sequence of the 7064-nt fragment subsequently was determined from BCNVL3 (3961-bp) and BCNVL4 (3792-bp), which were generated from the AV A0070039 L segment first-strand cDNA by using the Triple Master PCR System (Eppendorf) in conjunction with 19C-cons *and* AVPOL33 (5′-AGGTATGATCACCGAAGTAG-3′) and 19C-cons *and* AVPOL83 (5′-CCTGTCCATTAAGCCAAGCC-3′), respectively. BCNVL3 was ligated into the pGEM®-T Easy vector (Promega Corp., Madison, WI) and the sequence of 1 strand of the cloned BCNVL3 was determined. Multiple attempts to propagate plasmids that contained a BCNVL4 insert were unsuccessful; therefore, 1 strand of BCNVL4 was sequenced directly. The single base discrepancy between the BCNVL1-BCNVL2 sequence and the BCNVL3-BCNVL4 sequence was resolved by comparison with the homologous sequence of a 2196-bp product generated from the AV A0070039 L segment first-strand cDNA. The 2196-bp product was generated by using the Triple Master PCR System (Eppendorf) in conjunction with 19C-cons and AVPOL1.

The nucleotide sequence of the 7103-nt fragment of the L segment of TAMV strain W-10777 was determined from 2 overlapping PCR products. TAMVL1 (3148-bp) and TAMVL2 (4457-bp) were generated from the first-strand cDNA by using the Triple Master PCR System (Eppendorf) in conjunction with 19C-cons *and* AVPOL90 (5′-TACACATCRAGTGATGATGAGATC-3′) and 19C-cons *and* AVPOL100 (5′-CGGTAACCYCTTGAWCCRTCMACCC-3′), respectively. Both strands of TAMVL1 and TAMVL2 were sequenced directly.

The nucleotide sequence of the 7094-nt fragment of the L segment of WWAV strain AV 9310135 was determined from 2 overlapping PCR products. WWAVL1 (3142-bp) and WWAVL2 (4451-bp) were generated by using the Triple Master PCR System (Eppendorf) in conjunction with 19C-cons *and* AVPOL90 and 19C-cons *and* AVPOL100, respectively. Both strands of WWAVL1 and WWAVL2 were sequenced directly. In a preliminary analysis, the sequence of the 318-nt fragment of the RdRp gene of WWAV strain AV 9310135 published previously (Charrel et al., 2003) and deposited into the GenBank nucleotide sequence database under accession no. AY216516 was only 78% identical to the sequence of the homologous region of WWAVL2. To provide an assurance that WWAVL2 was from WWAV strain AV 9310135, the sequence of a PCR product generated from the N protein region of the AV 9310135 first-strand cDNA in this study was compared to a 616-nt sequence of the N protein gene of WWAV strain AV 9310135 published previously (Fulhorst et al., 1996) and deposited into the GenBank nucleotide sequence database under accession no. U52180. The sequence of a 616-nt fragment of the PCR product generated from the first-strand cDNA in this study was identical to the N protein gene sequence in GenBank accession no. U52180. Subsequently, total RNA was isolated directly from the virus stock that was used to infect the Vero E6 cells, a first-strand cDNA was generated from the arenavirus-specific by using SuperScript II RNase H^- Reverse Transcriptase (Invitrogen Life Technologies) in conjunction with 19C-cons, and a 1976-bp PCR product was generated from the L segment first-strand cDNA by using the Triple Master PCR System (Eppendorf) in conjunction with AVPOL96 (5′-AATAAGAGYGTTGTTGTCCC-3′) and AVPOL100. The sequence of this PCR product was

identical to the sequence of the homologous region of WWAVL2. Together, the sequence of the N protein gene generated from the original first-strand cDNA and the sequence of the 1976-nt fragment of the L segment generated from the virus stock indicate that the sequence of WWAVL2 is from WWAV strain AV 9310135.

The nucleotide sequence of the 7008-nt fragment of the L segment of AMAV strain BeAn 70563 was determined from 2 overlapping PCR products. AMAVL1 (3995-bp) and AMAVL2 (4576-bp) were amplified from the AMAV L segment first-strand cDNA by using the Triple Master PCR System (Eppendorf) in conjunction with 19C-cons *and* AVPOL70 (5′-AGATGTTTCGAAGAACAGG-3′) and 19C-cons *and* AVPOL77 (5′-GAGAGGTTGTGAAGTATTCC-3′), respectively. One strand of each PCR product was sequenced directly. Subsequently, AMAVL1 and AMAVL2 each were ligated into the pGEM®-T Easy vector (Promega Corp.) and 1 strand of the cloned AMAVL1 and 1 strand of the cloned AMAVL2 were sequenced.

The nucleotide sequence of the 3290-nt fragment of the S segment of BCNV strain AV 98470029 and the 3301-nt fragment of the S segment of BCNV strain AV A0070039 were deposited into the GenBank nucleotide sequence database under accession nos. AY924392 and AY924391, respectively. The nucleotide sequence of the 7064-nt fragment of the L segment of strain AV A0070039, the 7103-nt fragment of the L segment of TAMV strain W-10777, the 7094-nt fragment of the L segment of WWAV strain AV 9310135 and the 7008-nt fragment of the L segment of AMAV strain BeAn 70563 were deposited into the GenBank nucleotide sequence database under accession nos. AY924390, AY924393, AY924395 and AY924389, respectively.

Data analysis

The nucleotide sequences of the GPC and N protein genes of strain AV 98470029 were compared to the homologous sequences of BCNV strain A0060209, BCNV strain A0070039, TAMV strain W-10777, WWAV strain AV 9310135, TCRV strain TRVL II573 and the 7 South American arenaviruses included in the analyses of GPC and N protein gene sequence data (Table 2). Subsequently, the nucleotide sequences of the Z genes and RdRp genes of BCNV strain A0070039, TAMV strain W-10777 and WWAV strain AV 9310135 were compared to the homologous sequences of TCRV strain TRVL II573 and the 7 South American arenaviruses included in the analyses of Z and RdRp gene sequence data (Table 3). The GPC, N protein, Z protein and RdRp amino acid sequences were aligned independently, using the computer program Clustal W1.7 (Thompson et al., 1994). The nucleotide sequences were aligned manually based on the computer-generated amino acid sequence alignments. The GPC, N protein, Z and RdRp gene sequence alignments were 1683, 1734, 333 and 6942 characters in length, respectively. The analyses of the nucleotide sequence alignments were done by using programs in the computer software package PAUP*, version 4.0b10 (Swofford, 2003) and MEGA, version 2.1 (Kumar et al., 2001). Nucleotide sequence identities were calculated by subtracting uncorrected *p* model distances from 1.0. Bootstrap support (Felsenstein, 1985) for the results of the neighbor-joining analyses of *p* model distances and maximum parsimony analyses of nucleotide sequence data was based on analyses of 1000 pseudoreplicate datasets and 500 pseudoreplicate datasets, respectively. The topologies of the neighbor-joining phylograms and corresponding maximum parsimony phylograms were essentially identical. The LCMV strain WE (GenBank accession nos. M22138 and AF004519) and LASV strain Josiah (NC_004296 and NC_004297) were used as outgroup taxa in the neighbor-joining analyses to infer the ancestral node within the New World lineage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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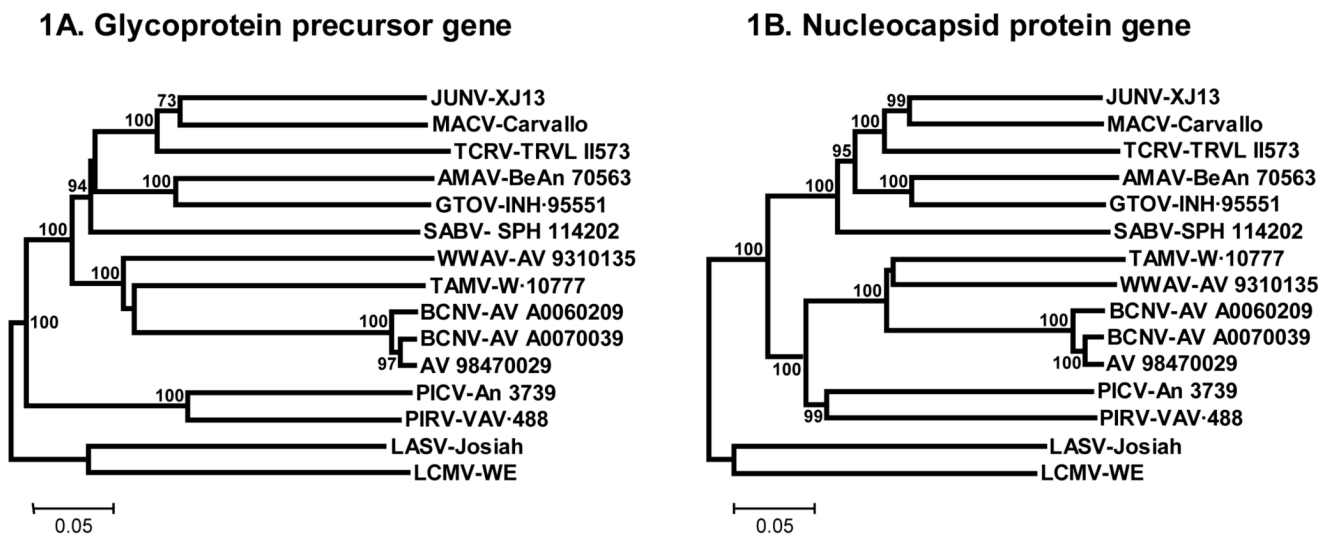

Figure 1.

Phylogenetic relationships among 15 arenaviruses based on neighbor-joining analyses of *p* model distances generated from alignments of (1A) complete glycoprotein precursor gene sequences and (1B) complete nucleocapsid protein gene sequences. The lengths of the horizontal branches are proportional to nucleotide sequence divergence. The length of each scale bar is equivalent to a sequence divergence of 0.05. The numerical value at the node indicates the percentage of 1000 bootstrap replicates that supported the interior branch. Bootstrap support values less than 70% are not listed. Strain AV 98470029 (GenBank accession no. AY924392); BCNV, *Bear Canyon virus* strains AV A0060209 and AV A0070039 (AF512833 and AY924390, respectively); TAMV, *Tamiami virus* strain W-10777 (AF512828); WWAV, *Whitewater Arroyo virus* strain AV 9310135 (AF228063); PICV, *Pichindé virus* strain An 3739 (NC_006447); PIRV, *Pirital virus* strain VAV-488 (NC_005894); AMAV, *Amapari virus* strain BeAn 70563 (AF512834); GTOV, *Guanarito virus* strain INH-95551 (NC_005077); JUNV, *Junin virus* strain XJ13 (NC_005081); MACV, *Machupo virus* strain Carvallo (NC_005078); SABV, *Sabiá virus* strain SPH 114202 (NC_006317); TCRV, *Tacaribe virus* strain TRVL II573 (NC_004293); LASV, *Lassa virus* strain Josiah (NC_004296); LCMV, *lymphocytic choriomeningitis virus* strain WE (M22138).

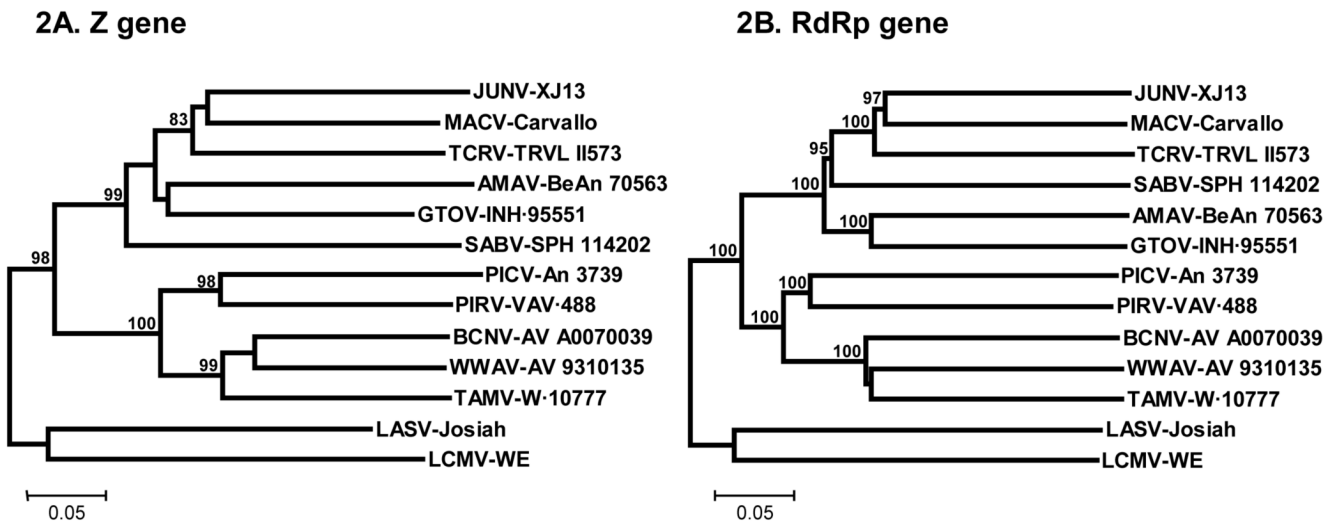

Figure 2.

Phylogenetic relationships among 13 arenaviruses based on neighbor-joining analyses of *p* model distances generated from alignments of (2A) complete Z gene sequences and (2B) complete RNA-dependent RNA polymerase gene sequences. The lengths of the horizontal branches are proportional to nucleotide sequence divergence. The length of each scale bar is equivalent to a sequence divergence of 0.05. The numerical value at the node indicates the percentage of 1000 bootstrap replicates that supported the interior branch. Bootstrap support values less than 70% are not listed. BCNV, *Bear Canyon virus* strain AV A0070039 (GenBank accession no. AY924391); TAMV, *Tamiami virus* strain W-10777 (AY924393); WWAV, *Whitewater Arroyo virus* strain AV 9310135 (AY924395); PICV, *Pichindé virus* strain An 3739 (NC_006439); PIRV, *Pirital virus* strain VAV-488 (NC_005897); AMAV, *Amapari virus* strain BeAn 70563 (AY924389); GTOV, *Guanarito virus* strain INH-95551 (NC_005082); JUNV, *Junin virus* strain XJ13 (NC_005080); MACV, *Machupo virus* strain Carvallo (NC_005079); SABV, *Sabiá virus* strain SPH 114202 (NC_006313); TCRV, *Tacaribe virus* strain TRVL II573 (NC_004292); LASV, *Lassa virus* strain Josiah (NC_004297); LCMV, *lymphocytic choriomeningitis virus* strain WE (AF004519).

Table 1

Histories of the 15 arenaviruses included in this study

Species[a]	Strain	Isolated from	
		Country (state)	Host[b]
Bear Canyon virus	AV A0060209	USA (California)	California mouse (*Peromyscus californicus*)
Bear Canyon virus	AV A0070039	USA (California)	California mouse (*P. californicus*)
Bear Canyon virus	AV 98470029	USA (California)	Large-eared woodrat (Neotoma macrotis)
Tamiami virus	W-10777	USA (Florida)	Hispid cotton rat (*Sigmodon hispidus*)
Whitewater Arroyo virus	AV 9310135	USA (New Mexico)	White-throated woodrat (*Neotoma albigula*)
Pichindé virus	An 3739	Colombia	Tomes's oryzomys (*Oryzomys albigularis*)
Pirital virus	VAV-488	Venezuela	Alston's cotton rat (*Sigmodon alstoni*)
Amapari virus	BeAn 70563	Brazil	Guiana bristly mouse (*Neacomys guianae*)
Guanarito virus	INH-95551	Venezuela	Human (*Homo sapiens*)
Junin virus	XJ13	Argentina	Human (*H. sapiens*)
Machupo virus	Carvallo	Bolivia	Human (*H. sapiens*)
Sabiá virus	SPH 114202	Brazil	Human (*H. sapiens*)
Tacaribe virus	TRVL II573	Trinidad	Frugivorous bats (*Artibeus spp.*)
Lassa virus	Josiah	Sierra Leone	Multi-mammate mouse (*Mastomys natalensis*)
Lymphocytic choriomeningitis virus	WE	USA	House mouse (*Mus musculus*)

[a] Strains AV A0060209 and AV A0070039 were isolated from California mice captured in June 1998 in Riverside County and in November 1998 in Orange County, respectively. Strain AV 98470029 was isolated from a large-eared woodrat captured in September 1998 in Riverside County.

[b] The principal hosts of Guanarito virus, Junin virus and Machupo virus are the short-tailed cane mouse (*Zygodontomys brevicauda*), drylands vesper mouse (*Calomys musculinus*) and large vesper mouse (*Calomys callosus*), respectively. The principal host of Sabiá virus has not been determined.

Table 2

Nucleotide sequence identities among the glycoprotein precursor genes and among the nucleocapsid protein genes of 13 New World arenaviruses[a]

	Glycoprotein precursor gene (% nucleotide sequence identity)											
Virus(es)[b]	**AV 98470029**	**BCNV**	**TAMV**	**WWAV**	**PICV**	**PIRV**	**AMAV**	**GTOV**	**JUNV**	**MACV**	**SABV**	**TCRV**
AV 98470029	--	97.0--98.1	64.0	62.5	50.6	51.9	55.4	55.6	57.3	57.2	56.7	53.6
BCNV	96.4--98.0	--	63.9--64.3	62.5--62.9	50.4--50.7	51.6--52.0	55.4--55.9	55.6--55.8	56.9--57.1	57.1--57.2	56.6--56.8	52.9--53.2
TAMV	71.8	71.8	--	61.4	49.2	50.0	55.8	55.1	55.6	55.2	56.6	54.2
WWAV	71.9	72.1--72.3	71.7	--	51.1	52.3	53.1	54.2	54.9	57.0	56.0	53.4
PICV	63.4	63.2--63.6	62.1	63.1	--	72.6	51.1	51.2	49.2	48.9	50.2	50.0
PIRV	63.9	63.7--63.9	62.0	62.2	66.7	--	51.5	50.9	50.5	51.0	52.4	52.2
AMAV	57.7	57.3	55.2	56.6	59.4	57.9	--	67.8	58.2	58.7	57.6	56.9
GTOV	57.3	57.5	57.1	57.9	59.2	59.0	75.3	--	57.9	57.9	58.7	56.4
JUNV	56.9	56.6	57.2	56.5	57.6	59.3	68.2	69.6	--	69.1	57.5	66.6
MACV	59.7	59.6--59.8	56.6	58.1	58.6	60.3	67.4	69.4	76.1	--	58.3	62.9
SABV	58.8	58.5--59.0	57.1	58.0	58.5	57.1	66.8	66.0	66.8	67.3	--	57.0
TCRV	57.8	57.9	57.0	57.0	59.0	58.4	67.6	67.7	72.2	71.5	64.8	--
	Nucleocapsid protein gene (% nucleotide sequence identity)											

[a] Sequence identities among the glycoprotein precursor genes and among the nucleocapsid protein genes are listed above and below the diagonal, respectively.

[b] Strain AV 98470029 (GenBank accession no. AY924392); BCNV, *Bear Canyon virus* strains AV A0060209 and AV A0070039 (AF512833 and AY924390, respectively); TAMV, *Tamiami virus* strain W-10777 (AF512828); WWAV, *Whitewater Arroyo virus* strain AV 9310135 (AF228063); PICV, *Pichindé virus* strain An 3739 (NC_006447); PIRV, *Pirital virus* strain VAV-488 (NC_005894); AMAV, *Amapari virus* strain BeAn 70563 (AF512834); GTOV, *Guanarito virus* strain INH-95551 (NC_005077); JUNV, *Junin virus* strain XJ13 (NC_005081); MACV, *Machupo virus* strain Carvallo (NC_005078); SABV, *Sabiá virus* strain SPH 114202 (NC_006317); TCRV, *Tacaribe virus* strain TRVL II573 (NC_004293). The nucleotide sequence of the GPC gene of BCNV strain AV A0060209 was 97.2% identical to the homologous sequence of BCNV strain AV A0070039 and the nucleotide sequence of the nucleocapsid protein gene of strain AV A0060209 was 96.3% identical to the homologous sequence of strain AV A0070039.

Table 3

Nucleotide sequence identities among the Z genes and among the RNA-dependent RNA polymerase genes of 11 New World arenaviruses[a]

	Z gene (% nucleotide sequence identity)										
Virus[b]	**BCNV**	**TAMV**	**WWAV**	**PICV**	**PIRV**	**AMAV**	**GTOV**	**JUNV**	**MACV**	**SABV**	**TCRV**
BCNV	-	73.3	75.1	60.4	60.7	48.4	53.0	46.4	48.9	48.1	47.3
TAMV	66.5	-	68.1	62.1	60.7	47.3	53.0	46.7	48.2	50.2	46.2
WWAV	67.9	67.7	-	62.8	62.1	48.4	52.7	44.9	47.1	50.2	49.8
PICV	56.5	56.6	56.8	-	68.1	48.7	46.2	44.2	46.7	45.3	44.4
PIRV	57.1	57.8	57.4	60.9	-	47.7	50.5	48.6	50.0	49.5	46.2
AMAV	50.2	50.3	50.5	51.0	51.7	-	64.2	57.8	61.0	54.0	61.1
GTOV	51.1	51.0	51.2	51.2	51.6	67.2	-	64.2	62.8	60.7	64.9
JUNV	50.4	49.7	49.8	51.0	50.8	60.6	61.1	-	68.1	55.3	65.6
MACV	51.4	50.8	50.6	51.3	51.4	61.0	61.4	68.8	-	60.6	67.7
SABV	51.0	51.2	50.8	50.4	51.0	61.2	60.7	61.9	61.6	-	58.2
TCRV	50.5	50.5	50.1	50.9	51.0	60.4	60.6	66.9	67.3	61.6	-
	RNA-dependent RNA polymerase gene (% nucleotide sequence identity)										

[a] Sequence identities among the Z genes and among the RdRp genes are listed above and below the diagonal, respectively.

[b] BCNV, *Bear Canyon virus* strain AV A0070039 (GenBank accession no. AY924391); TAMV, *Tamiami virus* strain W-10777 (AY924393); WWAV, *Whitewater Arroyo virus* strain AV 9310135 (AY924395); PICV, *Pichindé virus* strain An 3739 (NC_006439); PIRV, *Pirital virus* strain VAV-488 (NC_005897); AMAV, *Amapari virus* strain BeAn 70563 (AY924389); GTOV, *Guanarito virus* strain INH-95551 (NC_005082); JUNV, *Junin virus* strain XJ13 (NC_005080); MACV, *Machupo virus* strain Carvallo ((NC_005079); SABV, *Sabiá virus* strain SPH 114202 (NC_006313); TCRV, *Tacaribe virus* strain TRVL II573 (NC_004292).