Highly Diverse Arenaviruses in Neotropical Bats, Brazil

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We detected arenavirus RNA in 1.6% of 1,047 bats in Brazil that were sampled during 2007–2011. We identified Tacaribe virus in 2 *Artibeus* sp. bats and a new arenavirus species in *Carollia perspicillata* bats that we named *Tietê mammarenavirus*. Our results suggest that bats are an underrecognized arenavirus reservoir.

Bats are prominent hosts of zoonotic RNA viruses because of immunologic, physiologic, and ecologic factors (*1*). The Arenaviridae family comprises 4 genera: *Reptarenavirus* and *Hartmanivirus*, whose members infect reptiles; *Antennavirus*, whose members infect fish; and *Mammarenavirus*, whose members infect mammals. Mammarenaviruses can be separated into globally distributed lymphocytic choriomeningitis–Lassa virus serocomplex and New World arenaviruses (NWAs) (*2*). The NWAs Junin, Machupo, Sabia, Chapare, and Guanarito cause viral hemorrhagic fever and must be handled under Biosafety Level 4 conditions (*2*).

All highly pathogenic arenaviruses known thus far are hosted by and transmitted to humans from persistently infected rodents (*2*). Only Tacaribe virus (TCRV; *Tacaribe mammarenavirus*) has been identified in bats (*3*,*4*). Although TCRV is not considered a human pathogen, anecdotal evidence exists for potential laboratory acquired infection that causes influenza-like symptoms (*5*,*6*). In addition, TCRV is phylogenetically related to pathogenic arenaviruses that cause viral hemorrhagic fever; viral properties associated with severe disease, such as evasion of immune responses and cellular tropism, might be conserved in TCRV and genetically related animal arenaviruses (*7*).

Associations between TCRV and *Artibeus* spp. bats are supported only by limited epidemiologic data, including a single virus isolation and serologic evidence (*3*,*4*), considerable illness of bats during experimental infection (*5*), and isolation of TCRV from mosquitoes and ticks that primarily feed on rodents and rarely on bats (*3*,*6*). Limited genetic data exist for TCRV; a single genomic sequence was obtained from a bat-derived isolate generated in the 1950s from Trinidad that has been extensively passaged in mice and cell cultures and another from a recent tick-derived isolate (*3*,*4*,*8*).

The Study

We investigated diverse specimens from 1,047 adult bats belonging to 32 species collected from southeastern Brazil (Appendix, https://wwwnc.cdc.gov/EID/article/28/12/22-0980-App1.pdf). We analyzed a total of 3,670 different tissue specimens, including spleens (n = 893), lungs (n = 889), intestines (n = 973), and livers (n = 915), for arenavirus RNA by using reverse transcription PCR (RT-PCR) (*9*) modified to promote NWA amplification (Appendix Table 1, Figure 1).

We detected arenavirus RNA in 4 *Artibeus lituratus*, 1 *A. planirostris*, and 12 *Carollia perspicillata* bats; the overall detection rate was 1.62% (95% CI 0.95%–2.59%). Arenavirus-positive bats were collected during 2007–2011 from 3 sampling sites located in both forest and urban areas within a 60-km radius (Figure 1), suggesting arenavirus

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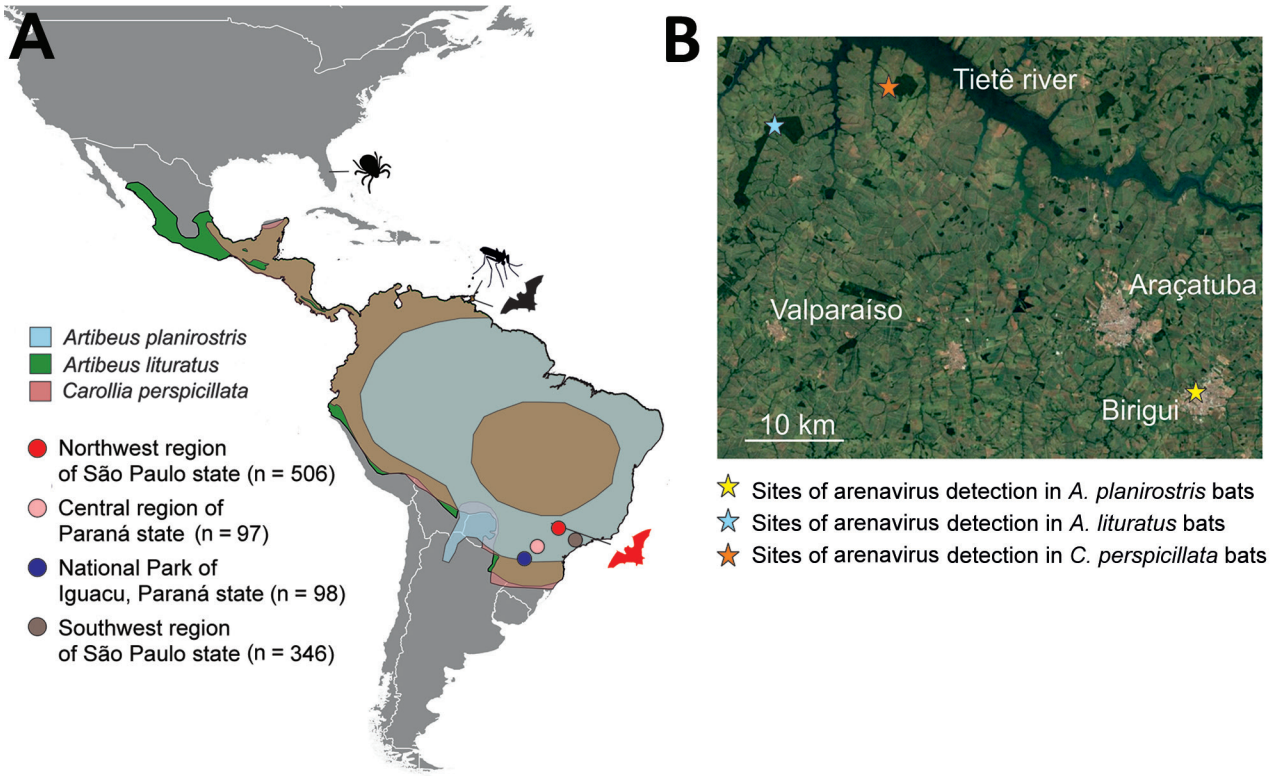

Figure 1. Bat mammarenavirus detection and host distribution in study of highly diverse arenaviruses in neotropical bats, Brazil. A) Geographic ranges of arenavirus-positive bat species indicated by blue (*Artibeus planirostris*), green (*A. lituratus*), and red (*Carollia perspicillata*) colors, according to the International Union for Conservation of Nature (https://www.iucnredlist.org). The brown areas in the map indicate the overlap of the distribution of *A. lituratus* and *C. perspicillata*. The absence of *A. planirostris* distribution in central Brazil likely represents lack of information regarding this species. Filled circles represent regions of sample collection: northwestern region of São Paulo state (red), central region of Paraná state (pink), National Park of Iguaçu, Paraná state (dark blue), and southwestern region of São Paulo state (gray). Number of bats obtained from each region is indicated. Red bat figure indicates where *Tacaribe mammarenavirus* and *Tietê mammarenavirus* were detected in the present study. Hosts from which Tacaribe virus was sequenced in other studies, including ticks (Florida, USA), mosquitoes, and bats (Port of Spain, Trinidad and Tobago) are indicated by black pictograms. Map prepared using QGIS desktop software version 3.24 (https://www.qgis.org). B) Areas of arenavirus detection in the northwestern region of São Paulo state, Brazil. Yellow star indicates the capture site of arenavirus-positive *A. planirostris*, blue star indicates the capture site of arenavirus-positive *A. lituratus*, and orange star marks the capture site of arenavirus-positive *C. perspicillata* bats. Tietê River and cities Araçatuba, Valparaíso, and Birigui are indicated. Dark green areas show forest fragments. Map obtained from Google Earth (https://earth.google.com)

maintenance in bat populations in this region. All 3 arenavirus-positive bat species are abundant in tropical environments and well-adapted to urban landscapes, indicating potential for dispersion and spillover to humans and other animals.

Most arenavirus-positive bats were collected in 2 forest fragments in 2007 (Tables 1, 2; Figure 1), where most bat species positive for arenavirus RNA were sampled. Whether high detection rates at those sites correspond to epizootics or sampling bias remains unknown.

All arenavirus-positive animals appeared healthy, suggesting limited negative effects of arenavirus infection on bat hosts. This observation was similar in rodent arenavirus hosts (*10*) and consistent with high TCRV seroprevalence in a serologic survey (*4*) but different from experimental TCRV infections (*5*), likely because of different routes and high doses used for infecting bats in laboratory settings. High seroprevalence and low arenavirus detection rates suggest that arenaviruses do not infect bats persistently, which is distinct from results for rodent arenavirus infections (*11*). Lack of persistence is important for public health because it indicates potential limitations of arenavirus shedding by bat hosts whose lifespan is $\leq$8–12 years (*12*).

We detected arenavirus RNA in multiple organs at similar concentrations, including spleens (mean, 1.2×10^7 RNA copies/mg) and lungs (mean, 6.4×10^6 RNA copies/mg) ($p = 0.53$ by Mann-Whitney U test) (Table 2), suggesting systemic infection similar to that observed in experimentally infected bats (*5*). We

observed the highest arenavirus RNA concentration in the single arenavirus-positive intestine specimen, followed by the spleen, lung, liver, and kidney in that animal (Table 2). High arenavirus RNA concentrations in intestines are consistent with virus shedding through the enteric route, which has been observed during experimental infections with TCRV (*5*). Although rodents shed arenaviruses primarily through urine and saliva, shedding also occurs in feces (*2*). Determining differences in arenavirus transmission routes between bats and rodents will require further investigation. We were unsuccessful isolating bat arenaviruses from organ homogenates despite repeated attempts (Appendix), likely because of tissue degradation under tropical conditions.

We performed phylogenetic analysis of the partial sequence for the arenavirus RNA-dependent RNA polymerase gene obtained from RT-PCR screening. We found 2 NWA clades in bats from Brazil: 1 clade for both *Artibeus* spp. and 1 clade for *C. perspicillata* bats (GenBank accession nos. ON648806–16) (Figure 2, panel A). We obtained complete arenavirus coding sequences from 1 *A. planirostris* and 3 *C. perspicillata* bats (GenBank accession nos. ON648817–24) by using Illumina-based deep sequencing (Illumina, https://www.illumina.com); genome organization was identical to other mammarenaviruses. Both arenaviruses formed a well-supported monophyletic clade with TCRV in sister relationship to Junin and Machupo viruses (Figure 2, panel B) and

Table 1. Bat species screened for arenaviruses in study of highly diverse arenaviruses in neotropical bats, Brazil

Bat species	Family	No. bats	No. positive (%, 95% CI)*	Region†	Sampling year (no. bats)
Artibeus fimbriatus	Phyllostomidae	3	0	A	2012 (3)
A. lituratus	Phyllostomidae	155	4 (2.6, 0.7–6.5)	A–D	2007 (8), 2010 (26), 2011 (46), 2012 (45), 2013 (4), 2014 (12), 2015 (16)
A. obscurus	Phyllostomidae	2	0	C, D	2013, 2015
A. planirostris	Phyllostomidae	9	1 (11.1, 0.3–48.3)	A–C	2010 (3), 2011 (2), 2012 (2), 2013 (1), 2014 (1)
Carollia perspicillata	Phyllostomidae	63	12 (19.1, 10.3–30.9)	A–D	2007 (18), 2010 (13), 2011 (18), 2012 (12), 2015 (2)
Chrotopterus auritus	Phyllostomidae	1	0	A	2010
Cynomops planirostris	Molossidae	11	0	C, D	2013 (1), 2014 (7), 2015 (3)
Desmodus rotundus	Phyllostomidae	69	0	C, D	2007 (7), 2011 (44), 2012 (1), 2014 (15), 2015 (2)
Eptesicus furinalis	Vespertilionidae	17	0	C, D	2011 (2), 2013 (6), 2014 (3), 2015 (6)
Eumops auripendulus	Molossidae	2	0	D	2014, 2015
E. glaucinus	Molossidae	106	0	C, D	2009 (1), 2010 (1), 2011 (5), 2012 (5), 2013 (19), 2014 (34), 2015 (41)
E. perotis	Molossidae	12	0	C, D	2013 (1), 2014 (8), 2015 (3)
Glossophaga soricina	Phyllostomidae	70	0	C, D	2007 (3), 2011 (2), 2012 (1), 2013 (2), 2014 (30), 2015 (32)
Lasiurus blossevillii	Vespertilionidae	2	0	C, D	2011, 2012
L. cinereus	Vespertilionidae	1	0	C	2013
L. ega	Vespertilionidae	2	0	C, D	2013, 2014
Molossops neglectus	Molossidae	1	0	D	2014
M. temminckii	Molossidae	2	0	C	2011
Molossus molossus	Molossidae	242	0	C, D	2007 (1), 2010 (1), 2011 (25), 2012 (16), 2013 (60), 2014 (84), 2015 (55)
M. rufus	Molossidae	160	0	C, D	2009 (11), 2010 (1), 2011 (20), 2012 (28), 2013 (48), 2014 (27), 2015 (25)
Myotis nigricans	Vespertilionidae	35	0	C, D	2011 (1), 2012 (4), 2013 (9), 2014 (6), 2015 (15)
M. riparius	Vespertilionidae	1	0	C	2013
Noctilio albiventris	Noctilionidae	2	0	C	2007 (2)
Nyctinomops laticaudatus	Molossidae	4	0	C, D	2011 (1), 2014 (2), 2015 (1)
N. macrotis	Molossidae	1	0	D	2014 (1)
Phyllostomus discolor	Phyllostomidae	2	0	D	2014 (2)
Platyrrhinus lineatus	Phyllostomidae	6	0	C, D	2014 (5), 2015 (1)
Promops nasutus	Molossidae	1	0	D	2014 (1)
Pygoderma bilabiatum	Phyllostomidae	1	0	D	2015 (1)
Sturnira lilium	Phyllostomidae	29	0	A, B, D	2010 (5), 2011 (9), 2012 (14), 2015 (1)
Tadarida brasiliensis	Molossidae	30	0	C, D	2014 (15), 2015 (15)
Vampyressa pusila	Phyllostomidae	1	0	B	2012 (1)
Not identified		4	0	C, D	2011(1), 2013 (2), 2014 (1)
Total	4	1,047	17 (1.6, 0.9–2.6)	A–D	2007–2015

*Number of bats with arenavirus RNA detected by PCR.
†Bats were collected from 36 sites within 4 main geographic regions of Brazil: A, Iguaçu National Park; B, central region of Parana state; C, northwest São Paulo state; and D, southwest São Paulo state.

Table 2. Collection sites and arenavirus RNA concentrations in different organs from bats in study of highly diverse arenaviruses in neotropical bats, Brazil*

Sample no.	Bat species†	Sex	Collection site	No. RNA copies/mg tissue				
				Spleen	Lung	Intestine	Liver	Kidney
Br56	*Artibeus lituratus*	M	Valparaiso	5.54×10^2	7.24×10^2	NA	NA	NA
Br57	*A. lituratus*	M	Valparaiso	NA	**3.14×10^2**	NA	NA	NA
Br58	*A. lituratus*	M	Valparaiso	**2.15×10^6**	**6.13×10^6**	NA	NA	NA
Br59	*A. lituratus*	M	Valparais	NA	**2.10×10^2**	NA	NA	NA
A354	*A. planirostris*	M	Birigui	**1.09×10^5**	**5.02×10^4**	**4.73×10^5**	**9.68×10^3**	**6.01×10^3**
Br61	*Carollia perspicillata*	F	Araçatuba	**4.73×10^4**	**1.17×10^3**	NA	NA	NA
Br62	*C. perspicillata*	F	Araçatuba	**1.99×10^7**	**6.96×10^7**	NA	NA	NA
Br63	*C. perspicillata*	F	Araçatuba	2.71×10^2	$8.61 \times 10^{\circ}$	NA	NA	NA
Br65	*C. perspicillata*	M	Araçatuba	2.23×10^1	2.88×10^2	NA	NA	NA
Br68	*C. perspicillata*	F	Araçatuba	2.35×10^1	Neg	NA	NA	NA
Br69	*C. perspicillata*	M	Araçatuba	**5.95×10^7**	**1.99×10^6**	NA	NA	NA
Br70	*C. perspicillata*	M	Araçatuba	**8.20×10^7**	**1.85×10^5**	NA	NA	NA
Br71	*C. perspicillata*	F	Araçatuba	5.37×10^3	5.38×10^2	NA	NA	NA
Br72	*C. perspicillata*	M	Araçatuba	4.70×10^2	6.11×10^1	NA	NA	NA
Br74	*C. perspicillata*	M	Araçatuba	**3.54×10^5**	**8.84×10^6**	NA	NA	NA
Br76	*C. perspicillata*	M	Araçatuba	**1.18×10^6**	**1.52×10^7**	NA	NA	NA
Br77	*C. perspicillata*	F	Araçatuba	Neg	1.81×10^2	NA	NA	NA

*Numbers in bold are samples used in arenavirus isolation attempts. NA, tissue not available; Neg, negative
†Samples were collected from *Artibeus lituratus* bats in forest areas of Valparaiso in 2007, *A. planirostris* bat in an urban area of Birigui in 2011, and *Carollia perspicillata* bats in forest areas of Araçatuba in 2007.

Ocozocoautla de Espinosa virus that was possibly responsible for a hemorrhagic fever outbreak in Mexico (Figure 2, panel C) (*13*). These results highlight the genetic relationship of those bat-associated arenaviruses with highly pathogenic NWAs (Appendix Table 2). Identical topology in phylogenetic reconstructions

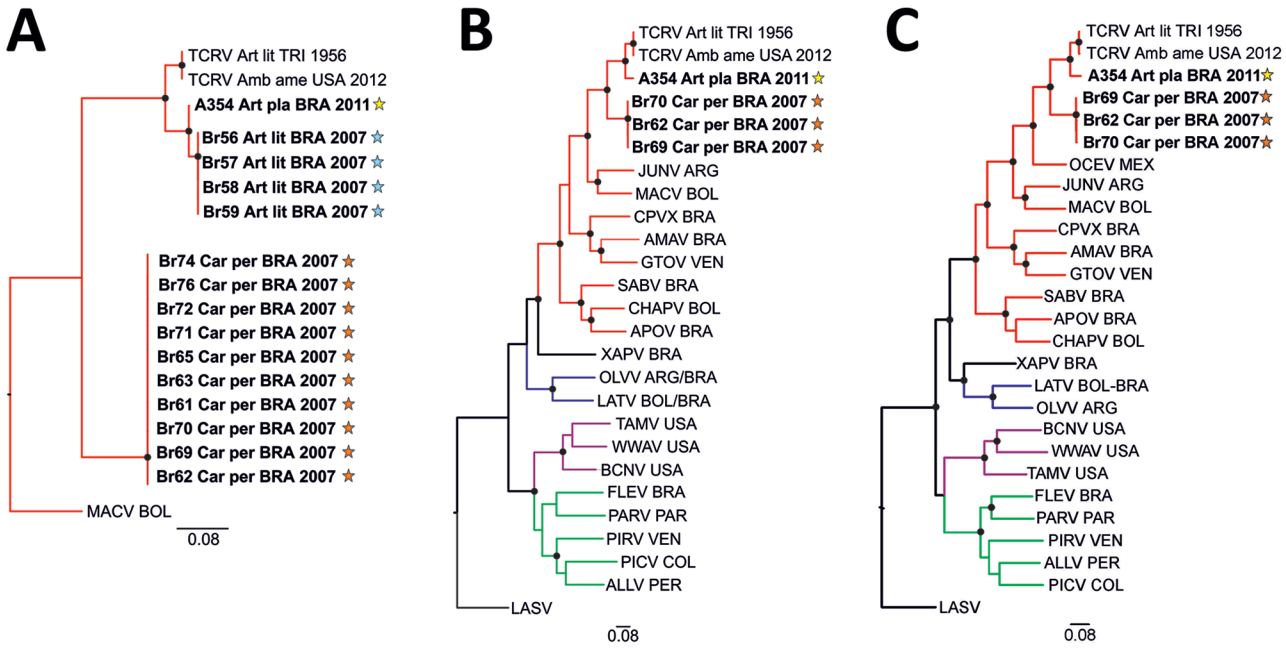

Figure 2. Phylogenetic analyses of highly diverse arenaviruses in neotropical bats, Brazil. Maximum-likelihood consensus trees compare partial RNA-dependent RNA polymerase genes (A), complete large (L) segment genes (B), and complete small (S) segment genes (C) from arenaviruses detected in *Artibeus* and *Carollia* spp. bats. Phylogenetic trees were generated using MEGA X software (https://www.megasoftware.net). Bold indicates sequences obtained from this study. Stars indicate regions where arenavirus-positive bat hosts were detected (Figure 1, panel B). Black dots at tree nodes represent bootstrap values ≥75% (1,000 replicates). Green lines indicate clade A new world arenaviruses, red lines indicate clade B new world arenaviruses, blue lines indicate clade C new world arenaviruses, and purple lines indicate recombinant new world arenaviruses (tentative clade D) (*14*). GenBank sequences used for comparisons and virus abbreviations are provided online (https://wwwnc.cdc.gov/EID/article/28/12/22-0980-F2.htm). Origins of arenaviruses are indicated for each sample: ARG, Argentina; BOL, Bolivia; BRA, Brazil; COL, Colombia; PER, Peru; TRI, Trinidad; USA, United States of America; VEN, Venezuela. Scale bars indicate nucleotide substitutions per site.

argued against potential reassortment (Figure 2, panels B, C), and homogeneous sequence distances and recombination analyses along the genome did not indicate recombination events (Appendix Figure 2).

The *A. planirostris* bat was infected with a previously unknown TCRV strain (Appendix Table 2) that had an amino acid identity of 93.8%–95.5% with other TCRV sequences, depending on the protein analyzed. The arenaviruses from *C. perspicillata* bats formed a separate species in clade B of the TCRV serogroup (Figure 2, panels B, C). Species assignment relied on taxonomic criteria (*14*) that included exclusive detection in a distinct host, nucleotide sequence identity of <80% in the small segment, and 88.6%–90% amino acid identity in the nucleocapsid protein compared with TCRV and pairwise sequence comparison (https://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage=overview) results for large and small segments (Appendix Figure 3). The 5′ and 3′ ends of large and small genomic segments obtained from the newly identified arenavirus from *C. perspicillata* bats were nearly identical to TCRV, consistent with a close genetic relationship between those NWAs (Appendix Table 3, Figure 4). We propose that the arenavirus sequenced from *C. perspicillata* bats should be named Tietê virus (species *Tiête mammarenavirus*) and abbreviated as TETV; the name comes from the main river located <4 km from the capture site (Figure 1).

Conclusions

Arenavirus genetic diversity is hypothesized to result from a complex macro-evolutionary pattern that includes both co-evolution and host switching in the Muridae family of rodents. In South America, arenaviruses might have co-evolved with rodents in the Sigmodontinae subfamily, with the exception of TCRV (*10*). Further investigation will be required to determine whether bat arenaviruses evolved from an ancestral host switch involving rodents, which would be consistent with the genetic relationship between TCRV or Tietê virus and rodent-derived Ocozocoautla de Espinosa virus, or whether bats and arenaviruses co-evolved. Of note, bats play an essential role in ecosystems, and stigmatization of bats as sources of zoonotic viruses is unwarranted.

In summary, the epidemiology, genealogy, and zoonotic potential of bat arenaviruses deserve further investigation. Our results suggest that bats are an underrecognized arenavirus reservoir.

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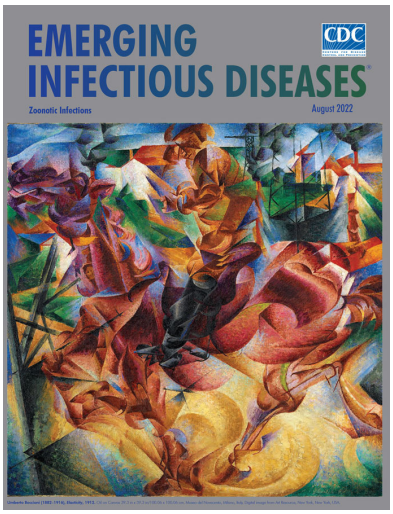

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- Child Melioidosis Deaths Caused by *Burkholderia pseudomallei*–Contaminated Borehole Water, Vietnam, 2019
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Highly Diverse Arenaviruses in Neotropical Bats, Brazil

Appendix

Additional Methods

Bat collection

Bats were collected from 36 sites during 2007–2015 that included pristine forest areas, forest fragments, and urban environments from 4 main geographic regions: National Park of Iguaçu, (south of Parana state characterized as a pristine area), central region of Parana state from 2 forest fragments, northwest region of São Paulo state (urban areas and forest fragments), and southwest region of São Paulo state (metropolitan areas and few urban forest fragments) (Figure 1). Bat species were identified by field biologists based on morphological criteria as described previously (*1*).

New World Arenavirus Screening Assay

We performed arenavirus screening by using broadly reactive primers (*2*) slightly modified according to alignments of New World arenavirus genomes from GenBank (Appendix Figure 2). Final primer sequences were: LVL_3359A_Mod, 5′-AGRCTTAGTGWGAGRGARAGCAAYTC-3′ and LVL_3754D_Mod, 5′-CWCATDATTGGICCCCAYTTWGARTGRTC-3′. We extracted viral RNA from 30 mg of bat tissue by using the MagNA Pure 96 DNA and Viral NA Large Volume Kit (Roche, https://www.roche.com). We screened samples by using SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Thermo Fisher Scientific, https://www.thermofisher.com). We performed PCR following the manufacturer's instructions in a final volume of 25 µL containing 5 µL RNA, 0.6 µmol/L primers, 12.5 µL 2× reaction mix buffer, 0.8 mmol/L $MgSO_4$, 1 µg bovine serum albumin, and 1 µL SuperScript III/Platinum Taq polymerase mix. Amplification was performed as follows: 50°C for 30 min followed by 95°C for

3 min; 45 cycles of 94°C for 15 s, 56°C for 20 s, and 72°C for 35 s; and final elongation at 72°C for 5 min. The primers generated an amplicon of 395 bp (*2*).

Virus Quantification

We quantified viruses by using one-step real-time RT-PCR with specific primers and probes for *Artibeus* sp. and *Carollia* sp. arenaviruses targeting the L gene: Art_Arena_Fwd, 5′-AACTGCCAACTGCCTCTGCA-3′; Art_Arena_Rev, 5′-CAAGGAGCAAGTGGGTTCCA-3′; Art_Arena_Probe, 5′-TGTTGAGATCCCCAACGTAAAGTTCCCT-3′; Car_Arena_Fwd, 5′-TTAGGTCCCCAACATACAGTTC-3′; Car_Arena_Rev, 5′-CACTAGCATCAGTTCAAATAATGG-3′; Car_Arena_Probe, 5′-AACCCACCTGTTCCTTATAACTCAAGCC-3′. We performed RT-PCR by using 25 µL reaction volumes containing 5 µL RNA, 2.0 mmol/L $MgCl_2$, 0.2 mmole/L (each) dNTPs, 0.4 µmol/L of each primer, 0.3 µmol/L probe, 1× PCR buffer, and 1 µL SuperScript III/Platinum Taq polymerase. We amplified the cDNA as follows: 50°C for 20 min, followed by 98°C for 3 min; 45 cycles of 98°C for 15 s, 58°C for 30 s (fluorescence was read at the 58°C step); and cooling at 40°C for 30 s. We quantified arenavirus RNA according to photometrically quantified cRNA transcribed from a gBlock synthetic gene fragment (Integrated DNA Technologies, Inc., https://www.idtdna.com) by using the MEGAscript T7 transcription kit (Thermo Fisher Scientific).

Virus Isolation Attempts

We attempted to isolate arenaviruses from arenavirus-positive organs of *Artibeus* sp. and *Carollia* sp. bats by using the cell lines Vero E6, C6/36, BHK-21, and CarLu/1 (lung epithelial cells isolated from a *Carollia perspicillata* bat) (*3*) and the immortalized primary cell line AJL derived from *Artibeus jamaicensis* lung tissue (*4*,*5*). Briefly, we shredded and homogenized 25 mg of arenavirus-positive tissue in 500 µL sterile 1× phosphate buffered saline at 30 Hz for 3 min by using a TissueLyser (QIAGEN, https://www.qiagen.com). To avoid cytotoxicity, we diluted the homogenates 5× and 50× in Leibovitz's L-15 Medium (C6/36 cells) or Dulbecco's Modified Eagle Medium (remaining cell lines) (Thermo Fisher, https://www.thermofisher.com) in 24-well plates 1 d before adding to cells. Cells were seeded at 1.5×10^5 (Vero cells), 2.5×10^5 (C6/36), 0.9×10^5 (BHK-21), 1.4×10^5 (CarLu), and 1.5×10^5 (AJL) cells/well. We prepared the cells in medium supplemented with 1% fetal bovine serum (Thermo Fisher Scientific) 1 h before

infection. After 1 h, we removed all medium and added a total of 200 µL homogenate to each well in a 24-well plate. The cells were incubated for 1 h at 37°C in 5% CO_2, except for C6/36, which were incubated for 1 h at 28°C without CO_2. We recovered the inoculum (200 µL) and stored for further experiments. We added 500 µL of fresh Leibovitz's L-15 Medium containing 5% fetal bovine serum, 1% penicillin-streptomycin, and 1% of 200 mM L-glutamine to the C6/36 cells and Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% nonessential amino acids to the other cells (Thermo Fisher Scientific). We observed the inoculated cells every 24 h to monitor cytopathic effects. We performed 5 serial blind passages every 5 d and collected supernatant samples (100 µL); cells were discarded if real-time RT-PCR results were negative for arenavirus.

Statistics

Confidence intervals were calculated by using Prism software (GraphPad, https://www.graphpad.com). We performed Mann-Whitney U tests using SPSS software, version 13.0 (IBM, https://www.ibm.com).

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Appendix Table 1. Bat species and tissues screened for arenavirus RNA in study of highly diverse arenaviruses in neotropical bats, Brazil*

Bat species	No. intestine	No. (%) positive	No. spleens	No. (%) positive	No. livers	No. (%) positive	No. lung	No. (%) positive	Total no. specimens
Artibeus fimbriatus	3	0	0	0	3	0	0	0	6
A. lituratus	145	0	100	2 (2.0)	95	0	64	4 (6.25)	404
A. obscurus	2	0	2	0	2	0	2	0	8
A. planirostris	8	1 (12.5)	8	1 (12.5)	9	1 (11.1)	7	1 (14.29)	32
Carollia perspicillata	45	0	40	11 (27.5)	31	0	34	12 (35.3)	150
Chrotopterus auritus	0	0	0	0	1	0	0	0	1
Cynomops planirostris	11	0	11	0	11	0	11	0	44
Desmodus rotundus	56	0	57	0	61	0	68	0	242
Eptesicus furinalis	17	0	14	0	17	0	17	0	65
Eumops auripendulus	2	0	2	0	2	0	2	0	8
E. glaucinus	103	0	103	0	102	0	106	0	414
E. perotis	12	0	12	0	12	0	12	0	48
Glossophaga soricina	66	0	66	0	67	0	70	0	269
Lasiurus blossevillii	2	0	2	0	2	0	2	0	8
L. cinereus	1	0	1	0	1	0	1	0	4
L. ega	2	0	2	0	2	0	2	0	8
Molossops neglectus	1	0	1	0	1	0	1	0	4
M. temmincki	2	0	2	0	2	0	2	0	8
Molossus molossus	239	0	227	0	238	0	239	0	943
M. rufus	145	0	153	0	148	0	160	0	606
Myotis nigricans	33	0	28	0	35	0	33	0	129
M. riparius	0	0	0	0	1	0	0	0	1
Noctilio albiventris	0	0	0	0	0	0	2	0	2
Nyctinomops laticaudatus	4	0	3	0	3	0	4	0	14
N. macrotis	1	0	1	0	1	0	1	0	4
Phyllostomus discolor	2	0	2	0	2	0	2	0	8
Platyrrhinus lineatus	6	0	6	0	6	0	6	0	24
Promops nasutus	1	0	1	0	1	0	1	0	4
Pygoderma bilabiatum	1	0	1	0	1	0	1	0	4
Sturnira lilium	29	0	15	0	23	0	5	0	72
Tadarida brasiliensis	30	0	29	0	30	0	30	0	119
Vampyressa pusila	0	0	0	0	1	0	0	0	1
Unknown	4	0	4	0	4	0	4	0	16
Total	973	1 (0.1)	893	15 (1.7)	915	1 (0.1)	889	17 (1.9)	3670

*Species in bold font had samples that were positive for arenavirus RNA.

Appendix Table 2. Comparisons of nucleotide and amino acid sequence distances of arenaviruses detected in *Artibeus planirostris* (sample A354) and *Carollia perspicillata* (sample Br70) bats in study of highly diverse arenaviruses in neotropical bats, Brazil*

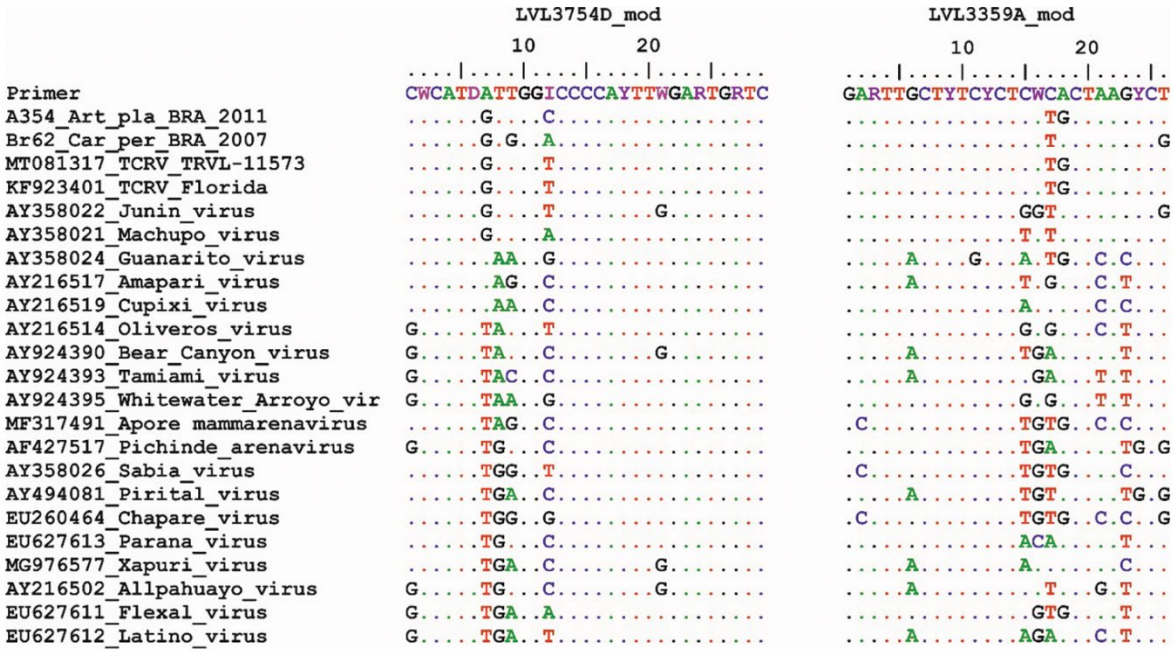
		L segment			S segment		
Sample	Arenavirus†	L segment (nt)	ZP (aa)	RdRp (aa)	S segment (nt)	GPC (aa)	NP (aa)
A354	TCRV TRVL	0.09	0.06	0.04	0.08	0.05	0.04
	TCRV USA	0.09	0.06	0.04	0.09	0.05	0.04
	OCEV	NA	NA	NA	0.27	0.25	0.17
	JUNV	0.33	0.29	0.29	0.30	0.32	0.20
	MACV	0.32	0.28	0.29	0.31	0.35	0.18
	Br70	0.23	0.17	0.15	0.21	0.13	0.10
Br70	TCRV TRVL	0.23	0.18	0.14	0.21	0.12	0.10
	TCRV USA	0.23	0.18	0.14	0.21	0.13	0.10
	OCEV	NA	NA	NA	0.26	0.25	0.16
	JUNV	0.33	0.28	0.30	0.30	0.29	0.20
	MACV	0.32	0.27	0.29	0.31	0.33	0.17
	A354	0.23	0.17	0.15	0.21	0.13	0.10

*Genetic distances were calculated by using MEGA X (MEGA, https://www.megasoftware.net). aa, amino acid; GPC, glycoprotein precursor; NA, sequence not available; NP, nucleocapsid protein; nt, nucleotide; RdRp, RNA-dependent RNA polymerase; ZP, zinc binding protein.
†GenBank sequences used for comparisons: TCRV TRVL, Tacaribe virus, strain TRVL-11573 L segment (MT081317) and S segment (MT081316.1); TCRV USA, Tacaribe virus, Florida isolate L segment (KF923401) and S segment (KF923400); OCEV, Ocozocoautla de Espinosa virus S segment (JN897398.1); JUNV, Junin virus L segment (AY358022) and S segment (NC_005081.1); and MACV, Machupo virus L segment (AY358021) and S segment (NC_005078.1).

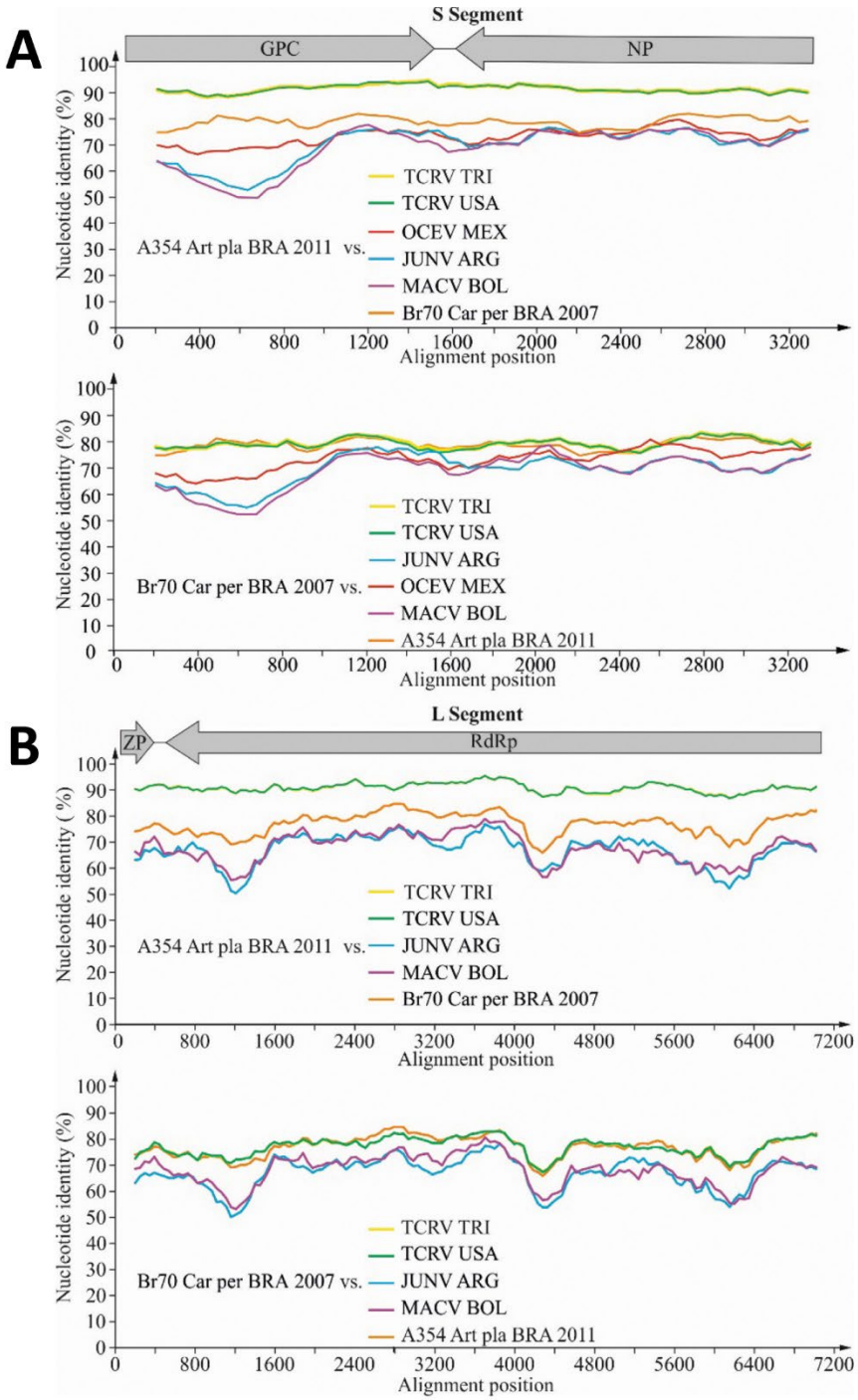
Appendix Table 3. Primers used to determine sequences of 5′ and 3′ ends of genomic segments L and S after rapid amplification of cDNA ends (RACE) in study of highly diverse arenaviruses in neotropical bats, Brazil*

Primer name	Primer sequence	Target segment
5′_RACE_A354_S_R3	CTCTTGCATGAAACTAATAAACTGC	S
5′_RACE_A354_S_R2	ATGCAATGTTGAGTGCTTCCTG	S
5′_RACE_A354_S_R1	TACTATGCAGATGAGGCTGACT	S
3`_RACE_A354_S_F1	AACGTCTGACTTGACTGTTTGG	S
3`_RACE_A354_S_F2	GAACTGACTCAATCCTTTTCTAAG	S
3`_RACE_A354_S_F3	GGCACTTCCTTGGATTGAGC	S
5′_RACE_A354_L_R3	TTCTGAGTTCGATTGCAGTTGC	L
5′_RACE_A354_L_R2	TGTGGTGGCTTCTCGAGATC	L
5′_RACE_A354_L_R1	CTTCTGAATTCTGCTGCTTGTG	L
3′_RACE_A354_L_F1	AAACCTTCTTGGAGTAATGATCCT	L
3′_RACE_A354_L_F2	CTGATGGGCAAACTCATGCC	L
3′_RACE_A354_L_F3	CAGGTCTTTGAGTTCAGAAACAG	L
5′_RACE_Car_S_R1	CATCTGTATAGGTTTACCAGACC	S
5′_RACE_Car_S_R2	TACAAGTGCGACATTGAGTGCT	S
5′_RACE_Car_S_R3	CTGCATGAAACTGATGAACTGC	S
3`_RACE_Car_S_F1	CATCTTTGAGAATATCTGACTTGAC	S
3`_RACE_Car_S_F2	GACTCAGCCCTTTCCTTAGG	S
3`_RACE_Car_S_F3	AAGCTGGGCACCTCTTTGGA	S
5′_RACE_Car_L_R1	CAACAGCACTTGCAGTTATAACG	L
5′_RACE_Car_L_R2	CTGCCACTCTTCTGAACTCC	L
5′_RACE_Car_L_R3	AGAGTAGAACAGACCTTTGAGC	L
3′_RACE_Car_L_F1	TAGGAGCTTGAAACCTTCTTGG	L
3′_RACE_Car_L_F2	TGAGTGACATTGTGACAAGAAGG	L
3′_RACE_Car_L_F3	TGTCTATTTGGAATGTGTTTCCTG	L

*Rapid amplification of cDNA ends was performed by using the 5′/3′ RACE Kit (Roche, https://www.roche.com) in accordance with the manufacturer's instructions.

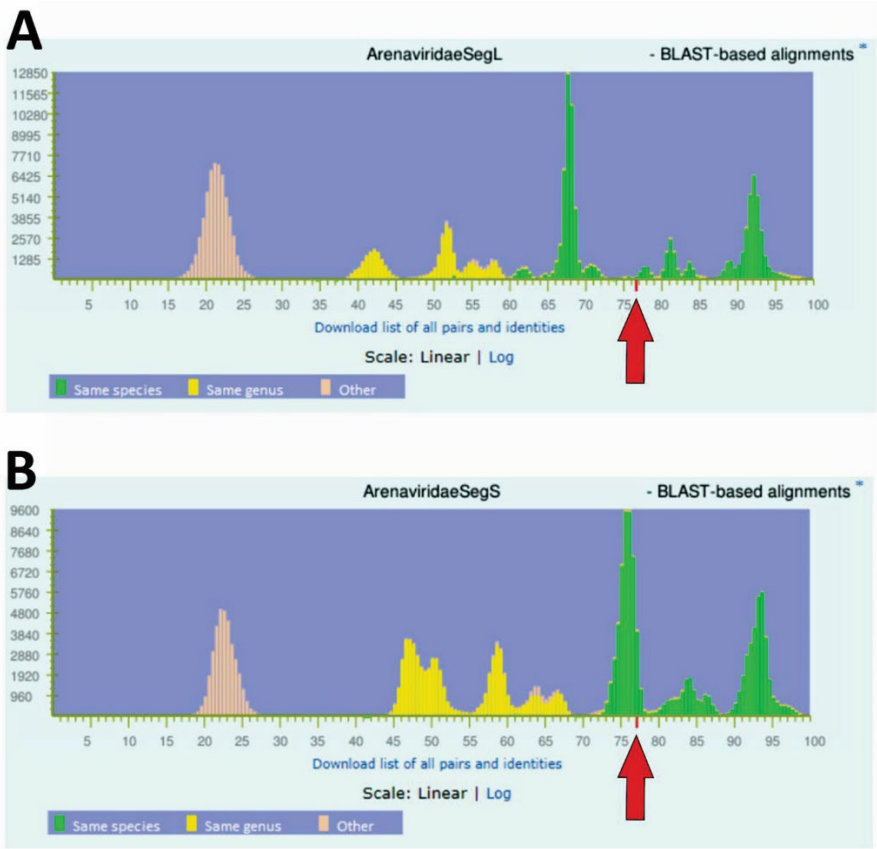


Appendix Figure 1. Comparison of binding sites of primers used for arenavirus screening between 23 L segment gene sequences from New World arenaviruses in study of highly diverse arenaviruses in neotropical bats, Brazil. The binding sites of primers LVL3754D_mod (5′–3′) and LVL 3359A_mod (3′–5′ genome binding site of primer is shown) were used as references. Nucleotide differences are indicated by letters; identical bases are shown as dots.

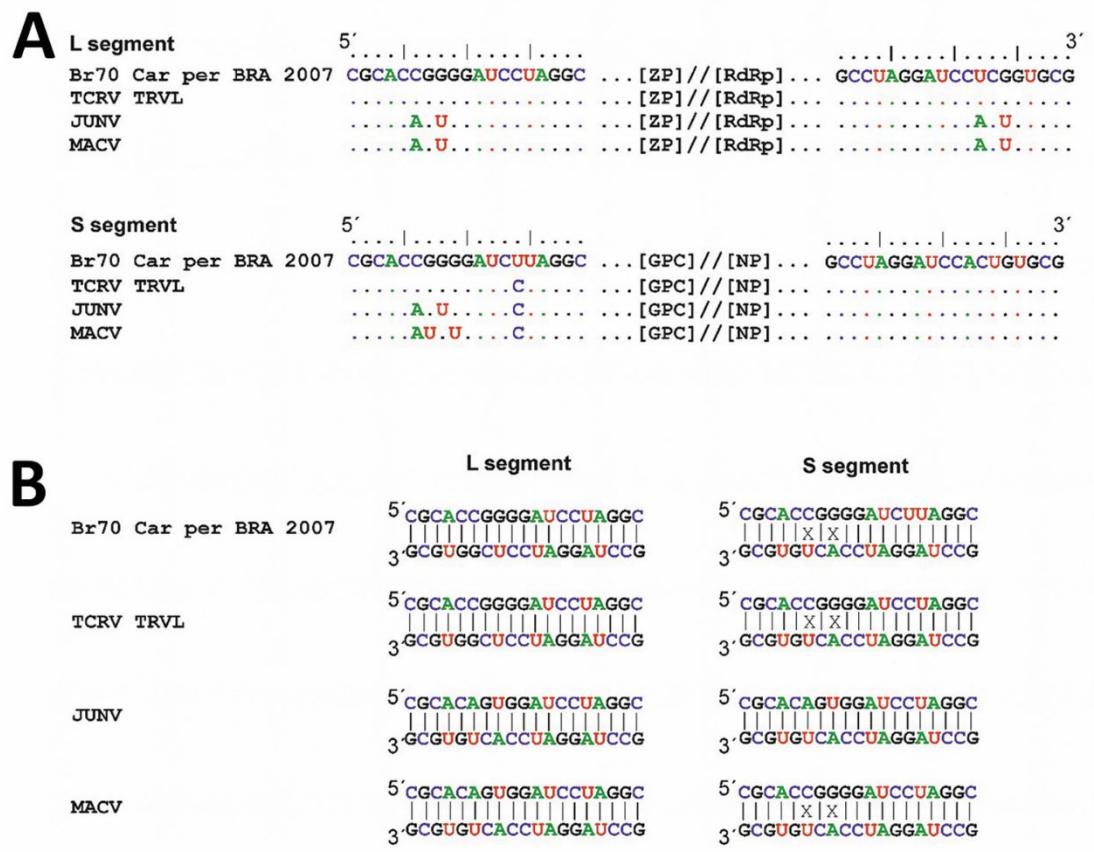


Appendix Figure 2. Genetic similarities between L and S segments of bat arenaviruses in study of highly diverse arenaviruses in neotropical bats, Brazil. We used SSE v1.4 software (http://www.virus-evolution.org/Downloads/Software) to generate genetic similarity plots to compare arenaviruses from *Artibeus planirostris* (sample A354) and *Carollia perspicillata* (sample Br70) bats with GenBank sequences for: TCRV_TRI, Tacaribe virus TRVL-11573 L segment (accession no. MT081317) and S segment (MT081316.1); TCRV USA, Tacaribe virus isolate Florida L segment (KF923401) and S

segment (KF923400); OCEV, Ocozocoautla de Espinosa virus S segment (JN897398.1) (L segment not available); JUNV, Junin virus L segment (AY358022) and S segment (NC_005081.1); and MACV, Machupo virus L segment (AY358021) and S segment (NC_005078.1). We used a 400 nt window with a 50 nt step (window was moved 50 nt in succession) and the p-distance model in SSE V1.4 software (http://www.virus-evolution.org/Downloads/software). Arenavirus genome organization includes 2 ambisense single-stranded RNA segments: a large (L) segment encoding RNA-dependent RNA polymerase (RdRp) and zinc binding protein (ZP) and a small (S) segment encoding the nucleocapsid protein (NP) and glycoprotein precursor (GPC). Recombination analyses were performed using the RDP4 program (http://web.cbio.uct.ac.za/~darren/rdp.html) and did not indicate recombinant events had occurred.



Appendix Figure 3. Sequence comparisons in study of highly diverse arenaviruses in neotropical bats, Brazil. We used the PAirwise Sequence Comparison (PASC) tool (https://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage=overview) to compare the A) L segment and B) S segment of the proposed new species *Tiête mammarenavirus* (sample Br70) with other members of the Arenaviridae family. Graphs already exist online for both gene segments in this virus family. The graphs show the percentage sequence identity of different family members on the x-axis and genome entries on the y-axis. We added our sequence to the program, and the red arrow indicates the percentage sequence identity of our sample with other viruses within the Arenaviridae family.



Appendix Figure 4. Analysis of genome termini of arenaviruses in study of highly diverse arenaviruses in neotropical bats, Brazil. We compared sequences of the 5′- and 3′-ends of L and S genomic segments obtained from the newly identified arenavirus (*Tiête mammarenavirus*) from *Carollia perspicillata* (specimen Br70 Car per BRA 2007) with those of other arenaviruses. A) Terminal sequences from Br70 Car per BRA 2007 were compared with the following GenBank reference sequences: TCRV-TRVL, Tacaribe virus TRVL-11573 L segment (accession no. MT081317) and S segment (MT081316.1); JUNV, Junin virus L segment (AY358022) and S segment (NC_005081.1), and MACV, Machupo virus L segment (AY358021) and S segment (KM198592.1). Identical bases are shown by dots. B) Complementarity was evaluated between the terminal 19 nts in the 5′- and 3′-untranslated regions of L and S segments. Mismatches are indicated by X, and base pairings by vertical lines. GPC, glycoprotein precursor; L, large segment; NP, nucleocapsid protein; RdRp, RNA-dependent RNA polymerase; S, small segment; ZP, zinc binding protein.