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Short Communication

Patawa Virus, a New Arenavirus Hosted by Forest Rodents in French Guiana

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Abstract: Molecular screening of rodents from French Guiana has detected a new arenavirus, named "Patawa," in two *Oecomys* species (Muridae, Sigmodontinae). Further investigations are needed to better understand the circulation of this virus in rodent and human populations and its public health impact.

Keywords: arenavirus, rodent, *Oecomys*, phylogeny, French Guiana, Amazonia

For decades, emerging viral diseases have become a major public health problem, with increasing occurrence of several diseases such as Ebola, SRAS, and Lassa fever. Three-quarters of emerging diseases are zoonotic and rodents are reservoirs for most of them (Woolhouse et al. 2001). In the Amazonian region, as in other tropical regions, these emergence phenomena are closely related to environmental disturbances inducing changes in richness, diversity, relative-specific abundance, and movements of animal communities (Confalonieri et al. 2014). These modifications profoundly alter the ecological dynamics at the edges of natural and anthropized habitats.

In French Guiana, 28 species of rodents are present, living in different habitats (various kinds of forests, savanna, agricultural areas, peri-urban, and urban zones). This French overseas department faces increasing environmental pressures, mainly on the coast where most of the population lives. This situation favors contacts between wild fauna and humans, with an associated increased risk of pathogen transmission (Berger et al. 2013; de Thoisy et al. 2014). Emerging viral diseases have recently appeared in the department: rabies in 2008 (Meynard et al. 2012), the first reported case of Hantavirus pulmonary syndrome in 2009 (Matheus et al. 2010) followed by three fatal cases in 2009, 2010, and 2013. To date, no human case of arenavirus has been registered in French Guiana. *Arenaviridae* constitutes a diverse family of enveloped single-stranded RNA viruses distributed worldwide. At least 29 arenavirus species are currently recognized (Charrel and de Lamballerie 2010). Some described in South America can induce, in severe cases, hemorrhagic fevers, but most are asymptomatic or cause relatively mild illnesses. They are mainly hosted by rodent species living in open areas. Only a few have been reported in the Amazonian forest region: Amapari, Flexal, and Cupixi viruses (Charrel et al. 2002; Milazzo et al. 2008). Investigating the presence of arenavirus in French Guiana, located in the Amazonian region, we implemented a survey on rodents captured in different habitats.

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Rodents were samples in 14 sites in French Guiana from 2001 to 2013. Sites were located in primary forests, edge and secondary forest habitats, savannas, and open habitats. The mean sampling effort by site was 1,016 ± 420 trap/nights, for a total of 28,670 trap/nights. Rodents were caught alive, brought to the laboratory, anesthetized and euthanized to collect their organs (kidney and lungs). The animals were identified using external morphological features and confirmed molecularly using Cytochrome Oxydase I sequence (Lim 2012). We captured and sampled 409 rodents (19 species) (Table 1). Total RNA was extracted from the organs and cDNA synthesized as previously described (de Thoisy et al. 2014). Presence of arenavirus was investigated by two nested RT-PCRs, using primers previously designed for amplification of a fragment of the Small (S) segment (661-bp of the nucleoprotein region (NP) using primer AREend-1696R and 1696R-1010C) (Bowen et al. 1996) and "screening" primers designed in this study to amplify a 431-bp fragment of the Large (L) segment (first-round PCR ARE-L3754F: GGAYCAYTCHAARTGGGGNCC–ARE-L4603R: CCATCNAYCCARTCTHTNACATC; second run PCR ARE-L4069F: TATGGGNCARGGVATNCTNCA–ARE-L4483R: ACACCATTNGCNACACAYTG). Amplification products were cloned using the TOPO-TA cloning system (Invitrogen, Cergy-Pontoise, France), sequenced, and consensus sequences were generated from three independent clones.

Table 1. Molecular Evidence of Arenavirus Infection in Rodents, in French Guiana, Based on the Amplification of the 661-bp NP and 431-L Fragments

Species	N pos/N tested
Holochilus sciureus	0/4
Makalata didelphoides	0/9
Mesomys hispidus	0/13
Mus musculus	0/33
Neacomys paracou	0/2
Nectomys rattus	0/5
Oecomys auyantepui	1/14
Oecomys bicolor	0/33
Oecomys rex	0/2
Oecomys rutilus	2/2
Oligoryzomys fulvescens	0/6
Oryzomys megacephalus	0/14
Oryzomys yunganus	0/10
Proechimys cayennensis	0/140
Proechimys cuvieri	0/47
Rattus rattus	0/19
Rhipidomys nitela	0/7
Sciurus aestuans	0/1
Zygodontomys brevicauda	0/48
Total	3/409

Taxonomy follows Wilson and Reeder (2005).
Mus musculus and *Rattus rattus* were caught in or very near houses in human settlements (isolated houses and/or villages).

Among the 409 rodents, three were found positive for both assays: two originated from *Oecomys rutilus* (field numbers V-2789 and V-3072) and one from *Oecomys auyantepui* (V-2790) (Table 1). These three arboreal mice (Muridae, Sigmodontinae) were caught by traps affixed to lianas 1.5–2.0 m above ground in a well-drained primary forest near the village of Cacao. V-2789 and V-2790 were caught in July 2010 approximately 100 m from each other, while V-3072 was caught 2 years later at around 50 m from the two previous rodents. The specimens were preserved as vouchers at the Museum d'Histoire Naturelle de Genève (Switzerland) under accessions MHNG-1975-049, MHNG-1975-050, and MHNG-1983-042 for V-2789, V-2790, and V-3072, respectively. The three specimens have been sequenced using the barcoding Cytochrome Oxydase 1 gene: the sequences corresponded to sequence reference of *Oecomys rutilus* (V-2789 and V-3072) and *O. auyantepui* (V-2790) according to Borisenko et al. (2008) and Lim (2012). Sequences have been deposited in GenBank under accession number KM102736–KM102738. In French Guiana, both species are found in primary forests and *O. auyantepui* is also not rare in secondary and slightly disturbed forests. These two species can sometimes be found in isolated human settlements (Catzeflis 2012). Arenavirus sequences from these three animals were obtained for the S and L segments (615- and 390-bp analyzed, respectively). Sequences of the S segment obtained for the two rodents captured in the same period showed 99.8 and 99.5% nucleotide (nt) and amino acid (aa) identity, respectively. The third sequence, identified from an *O. rutilus* captured 2 years later at the same site, diverged by about 10% at the aa level from the first two. In addition, comparison of these sequences with previously published arenaviruses revealed that they showed a high percentage of divergence with the other members of this viral family (Table 2), presenting between 68.5 and 66.9% nt identity with the most closely related Black Mesa virus and 61.4–66% aa identity with viruses of the recombinant New World group (Table 2). Considering the L "screening" fragment, arenavirus from French Guiana showed 81% aa identity with Bear Canyon virus and 98.3–100% aa identity between themselves (131-aa analyzed).

Table 2. Nucleotide (nt) and Amino Acid (aa) *p*-Distances Between Arenaviruses Found in Three Animals Belonging to *Oecomys* Genus and Arenaviruses Representative of the Different Lineages of New World Arenavirus and Old World Arenavirus According to Charrel and de Lamballerie (2010)

Lineages	Virus species	S segment nucleoprotein								S segment glycoprotein				L segment polymerase			
		1696/1010C						1696/ARE-end		AREend/S1a				L3509/L4603R			
		V-2790		V-2789		V-3072		V-2790		V-2790		V-3072		V-2790		V-3072	
		KJ668826		KJ668823		KJ668827		KJ668826		KJ668824		KJ668828		KJ668825		KJ668829	
		nt	aa	ntc	aa	nt	aa	ntc	aa	nt	aa	nt	aa	nt	aa	nt	aa
Old World viruses	Ippy virus NC_007905	52.6	41.6	52.6	41.1	50.8	41.6	56	51.6	45.1	33.5	44.5	33.5	44.7	36.1	44.7	35.3
	Lassa virus NC_004296	53.8	43.1	53.8	42.6	50.9	42.1	56.9	51.6	42.4	33.5	42.6	33.5	47.0	35.5	46.0	35.3
Lineage B New World viruses	Amapari virus AF485256	58.3	58.4	58.2	57.9	59.7	56.9	58.9	60.9	46.0	32.3	46.1	32.3	53.4	48.8	54.0	48.3
	Junin virus NC_005801	59.5	54.3	59.5	53.8	61.6	54.3	59.0	60.1	44.0	32.7	44.0	33.1	52.3	48.3	52.6	48.5
	Chapare virus NC_010562	59.9	58.4	59.7	57.9	61.0	59.9	59.0	60.5	47.7	35.7	48.3	35.7	52.8	48.5	52.8	48.3
	Cupixi virus NC_010254	59.9	55.8	59.9	55.3	57.5	54.8	59.3	59.9	44.5	31.6	44.4	31.2	53.3	52.0	54.9	52.0
	Ocozocoautla de Espinosa JN897398	59.7	54.8	59.5	54.3	59.7	54.3	59.1	60.3	43.1	30.5	43.5	29.7	–	–	–	–
	Sabia virus NC_006317	59.5	58.4	59.4	57.9	60.5	57.4	59.3	61.1	47.5	35.3	46.2	34.6	51.5	46.9	52.1	46.2
	Guanarito virus NC_006317	60.0	58.4	59.9	57.9	60.2	57.9	61.0	62.7	47.0	32.7	47.7	33.5	55.0	53.8	54.6	51.7
	Machupo virusNC_005078	60.9	54.8	60.7	54.3	60.7	53.8	61.1	60.9	43.6	31.2	43.7	30.9	53.8	48.3	55.3	49.6
	Tacaribe virus NC_004293	58.0	51.5	57.8	51.0	56.8	50.5	59.7	59.9	44.7	32.3	46.0	32.3	52.2	49.3	52.3	48.3
Lineage C New World viruses	Latino virus NC_010758	57.5	54.8	57.5	54.3	59.7	55.3	60.1	59.9	54.5	49.8	52.8	48.7	56.0	51.5	54.5	51.5
	Oliveros virus NC_010248	56.7	52.3	56.5	51.8	58.0	51.8	61.3	59.9	51.1	48.3	52.7	49.8	54.0	50.9	53.0	49.6
Recombinant New World viruses	Tamiami virus NC_010701	63.4	62.4	63.2	61.9	66.6	61.4	63.2	64.7	46.5	35.7	45.5	33.8	62.8	**65.5**	61.9	64.7
	Catarina virus DQ865245	63.9	64	63.7	63.5	65.6	**66.0**	65.3	68.8	47.1	34.2	46.5	32.3	–	–	–	–
	Big brushy tank virus EF619035	65.8	64.5	65.6	64.0	64.9	64.5	65.4	70.0	46.6	36.4	46.1	35.3	–	–	–	–
	Bear Canyon virus NC_010256	64.9	63.5	64.8	62.9	64.6	64.5	65.5	69.4	46.8	33.1	47.1	32.7	**63.6**	65.0	**64.1**	**65.5**
	Whitewater Arroyo virus NC_010700	65.6	65.5	65.4	65.0	64.9	**66.0**	65.8	69.4	46.6	32.0	46.6	32.3	60.7	63.4	61.5	63.9
	Skinner Tank virus EU123328	67.1	63.5	66.9	62.9	65.8	64.5	66.6	69.0	46.7	36.4	48.0	35.7	–	–	–	–
	Tonto creek virus EF619034	65.4	65	65.3	64.5	65.8	**66.0**	66.4	70.0	47.2	34.6	46.3	33.5	–	–	–	–
	Black Mesa virus FJ032027	**68.6**	**65.5**	**68.5**	**65.0**	**66.9**	65.0	66.7	69.6	48.7	32.7	47.7	32.7	–	–	–	–
Lineage A New World viruses	Pirital virus NC_005894	60.5	60.4	60.4	59.9	61.9	61.4	62.4	68.1	68.6	75.5	68.8	73.6	57.9	53.8	57.4	55.2
	Flexal virus NC_010757	63.2	64.5	63.2	64.0	64.4	64.5	66.1	70.8	73.1	82.9	72.6	81.4	56.9	58.4	59.3	57.0
	Parana virus NC_010756	65.9	65.5	65.8	65.0	65.9	65.5	66.6	**71.8**	71.7	80.7	71.9	79.2	57.7	55.7	59.5	56.8
	Allpahuayo virus NC_010253	64.8	60.9	64.6	60.4	64.1	61.4	**66.9**	69.4	**73.7**	**85.4**	**74.2**	**82.9**	56.5	56.0	56.2	56.2
	Pichinde virus NC_006447	63.9	64.0	63.7	63.5	63.9	64.5	**66.9**	70.6	70.8	75.8	70.1	74.3	57.6	56.0	57.4	55.2

Table 2. continued

Lineages	Virus species	S segment nucleoprotein							S segment glycoprotein				L segment polymerase			
		1696/1010C						1696/ARE-end	AREend/S1a				L3509/L4603R			
		V-2790		V-2789		V-3072		V-2790	V-2790		V-3072		V-2790		V-3072	
		KJ668826		KJ668823		KJ668827		KJ668826	KJ668824		KJ668828		KJ668825		KJ668829	
		nt	aa	ntc	aa	nt	aa	ntc	aa	nt	aa	nt	aa	nt	aa	nt	aa
This study	Patawa virus V-3072	77.2	91.4	77.1	90.9	–	–	–	–	79.4	93.3	–	–	77.7	86.7	–	–
	Patawa virus V-2789	99.8	99.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–

The highest identity value is indicated in bold. Accession numbers for the different amplified segments are indicated under the identification number of the animals from which they have been detected.

To further investigate the evolutionary relationships of this new virus with other members of the family, we attempted to sequence longer fragments of the S and L segments directly from the rodent specimens. We only succeeded in amplifying partial nucleoprotein (NP) and glycoprotein (GP) sequences located on the S segment (1,548-nt for NP and 963-nt for GPC) and a 1,214-nt fragment of the polymerase (L) gene for some of the strains identified in the different animals (Table 2). All viral sequences have been submitted to GenBank (accession numbers KJ668823–KJ668829 and KM233411). The sequence obtained for the NP fragment showed 71.8% aa identity with Parana virus, whereas GP-sequences showed 82.9–85.4% aa identity with Allpahuayo virus, both viruses belonging to lineage A (Table 2). Considering sequences of the L segment, arenavirus from French Guiana revealed around 65% identity with viruses belonging to the Recombinant New World clade (Tamiani virus and Whitewater Arroyo virus) (Table 2).

Phylogenetic relationships were inferred from alignment of the partial nucleotide sequences for the NP, GPC, and L fragments using a Bayesian approach performed with Mr. Bayes 3.2.2 (Ronquist et al. 2012). The partial sequence of the NP segment that was obtained from the *O. auyantepui* (V-2790) clusters with the recombinant New World monophyletic clade at the basal position of this group, with a high posterior probability value (0.99) (Fig. 1). Similar phylogenetic relationships were observed by analyzing the partial sequence of the L gene obtained from *O. auyantepui* (V-2790) and *O. rutilus* (V-3072) (Fig. 2). Partial sequences of the GPC segment obtained from two animals (*O. auyantepui* V-2790 and *O. rutilus* V-3072) cluster together and are related, within clade A of New World viruses, to Allpahuayo virus with a high posterior probability value (Fig. 3).

Screening 409 rodents captured in French Guiana for the presence of arenaviruses has identified two strains of a new arenavirus in two species of the *Oecomys* genus. Phylogenetic analyses on GPC and NP genes indicate that these viruses are closely related to Allpahuayo virus, which is also hosted by *Oecomys*. This rodent genus, widely distributed in the Amazonian basin, may therefore be a privileged reservoir of arenavirus in South America. Pairwise comparisons of amino acid sequences of the NP fragment suggest that these viruses belong to a novel arenavirus species (Salvato et al. 2005). After the hantavirus Maripa also detected in wild rodents (de Thoisy et al. 2014), we tentatively named this new arenavirus "Patawa" virus

Figure 1. Phylogenetic analysis of New World arenaviruses, including Patawa virus, based on partial nucleotide sequences of the NP (1,548-bp) gene. The GTR model, with gamma distribution (*G*) and proportion of invariable sites (*I*), was identified as the optimal model of nucleotide evolution (MrModeltest 2.2) (Nylander 2004). Phylogeny was inferred using Mrbayes software. Markov Chain Monte Carlo (MCMC) simulations were run for 10,000,000 generations, with four simultaneous chains, using a sample frequency of 500 and 25% burn-in. Virus names are associated with their accession numbers. The Patawa virus sequence generated in this study is in *boldface*. Associated lineages of New World arenaviruses correspond to those described in Charrel and de Lamballerie (2010). Support for nodes is provided by the posterior probabilities of the corresponding clades. All resolved nodes have posterior probability greater than 0.7. Scale bar indicates nucleotide sequence divergence among arenavirus sequences. Focus corresponds to the phylogenetic tree based on 615-bp of the NP diagnostic segment of the Patawa virus detected in the three rodents that belong to two different species of *Oecomys*.

(“Maripa” and “Patawa” are the common names for the *Attalea maripa* and *Oenocarpus bataua* palm trees, respectively, both widespread in the Guianan region). Its phylogenetic position, close to clade A or A/recombinant viruses that are usually less symptomatic, and the absence of reported cases of hemorrhagic fever in French Guiana suggest that it may not cause severe disease. Serological surveys in rodents and in the humans living in the area where the animals were captured could allow determining its circulation and its public health risks.

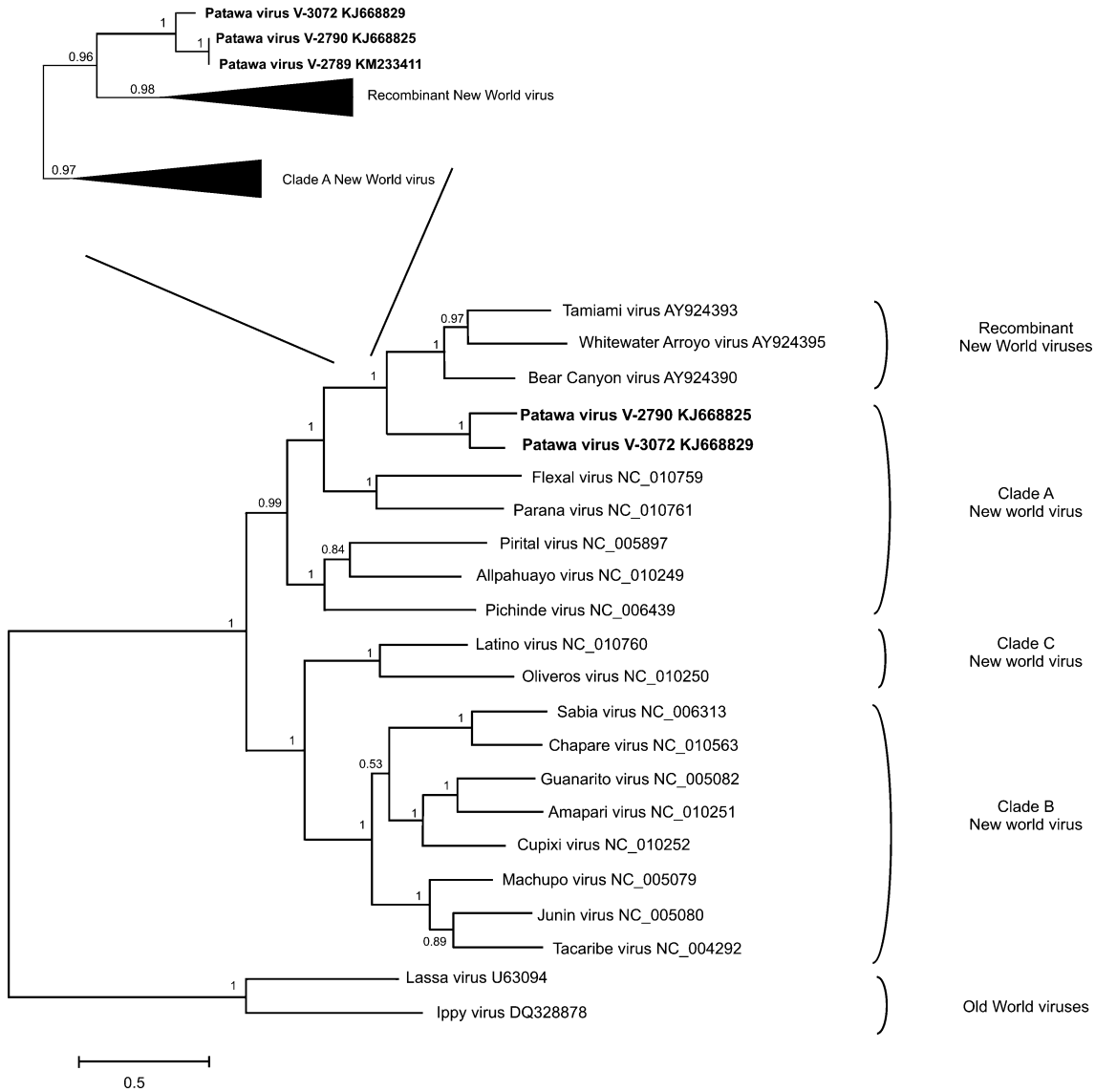

Figure 2. Phylogenetic analysis of New World arenaviruses, including Patawa virus, based on partial nucleotide sequences of the L (1,214-bp) gene. The GTR model, with gamma distribution (*G*) and proportion of invariable sites (*I*), was identified as the optimal model of nucleotide evolution (MrModeltest 2.2) (Nylander 2004). Phylogeny was inferred using Mrbayes software. Markov Chain Monte Carlo (MCMC) simulations were run for 10,000,000 generations, with four simultaneous chains, using a sample frequency of 500 and 25% burn-in. Virus names are associated with their accession numbers. The Patawa virus sequence generated in this study is in *boldface*. Associated lineages of New World arenaviruses correspond to those described in Charrel and de Lamballerie (2010). Support for nodes is provided by the posterior probabilities of the corresponding clades. All resolved nodes have posterior probability greater than 0.7. *Scale bar* indicates nucleotide sequence divergence among arenavirus sequences. Focus corresponds to the phylogenetic tree based on the 390-bp of the NP diagnostic segment of the Patawa virus detected in the three rodents belonging to two different species of *Oecomys*.

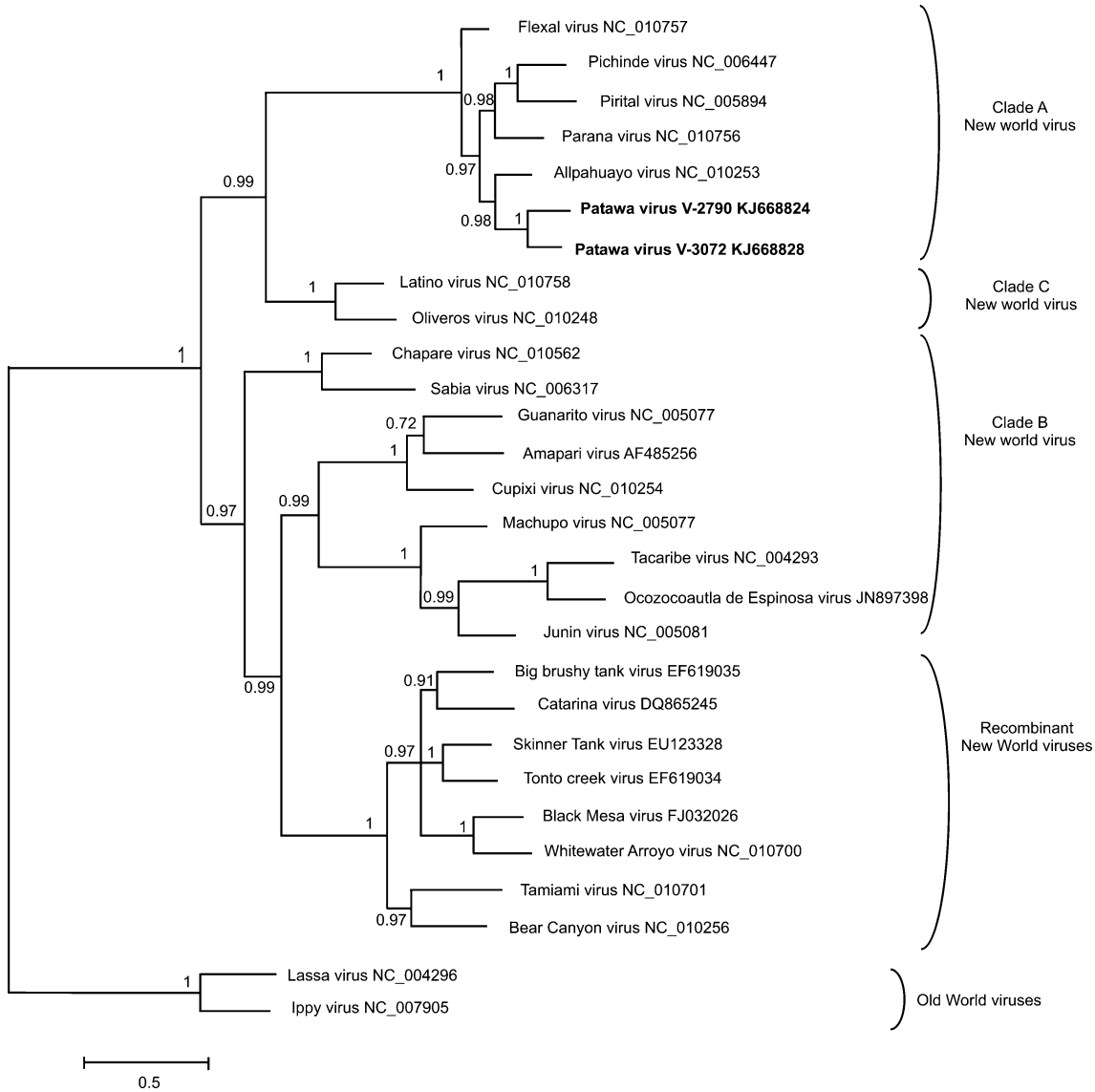

Figure 3. Phylogenetic analysis of New World arenaviruses, including Patawa virus, based on partial nucleotide sequences of the GPC (963 bp) gene. The GTR model, with gamma distribution (*G*) and proportion of invariable sites (*I*), was identified as the optimal model of nucleotide evolution (MrModeltest 2.2) (Nylander 2004). Phylogeny was inferred using Mrbayes software. MCMC simulations were run for 10,000,000 generations, with four simultaneous chains, using a sample frequency of 500 and 25% burn-in. Virus names are associated with their accession numbers. The Patawa virus sequences generated in this study are in *boldface*. Associated lineages of New World arenaviruses correspond to those described in Charrel and de Lamballerie (2010). Support for nodes is provided by the posterior probabilities of the corresponding clades. All resolved nodes have posterior probability greater than 0.7. *Scale bar* indicates nucleotide sequence divergence among arenavirus sequences.

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References

Berger F, Desplanches N, Baillargeaux S, Joubert M, Miller M, Ribadeau-Dumas F, et al. (2013) Rabies risk: difficulties encountered during management of grouped cases of bat bites in 2 isolated villages in French Guiana. *PLoS Neglected Tropical Diseases* 7:e2258

Borisenko AV, Lim BK, Ivanova NV, Hanner RH, Herbert PDN (2008) DNA barcoding in surveys of small mammal communities: a field study in Suriname. *Molecular Ecology Resources* 8:471–479

Bowen MD, Peters CJ, Nichol ST (1996) The phylogeny of New World (Tacaribe complex) arenaviruses. *Virology* 219:285–290

Catzeflis F (2012) A survey of small non-volant mammals inhabiting Wayampi Amerindian houses in French Guiana. *Mammalia* 76:327–330

Charrel RN, de Lamballerie X (2010) Zoonotic aspects of arenavirus infections. *Veterinary Microbiology* 140:213–220 . doi:10.1016/j.vetmic.2009.08.027

Charrel RN, Feldmann H, Fulhorst CF, Khelifa R, de Chesse R, de Lamballerie X (2002) Phylogeny of New World arenaviruses based on the complete coding sequences of the small genomic segment identified an evolutionary lineage produced by intrasegmental recombination. *Biochemical and Biophysical Research Communications* 296:1118–1124

Confalonieri UEC, Margonari C, Quintão AF (2014) Environmental change and the dynamics of parasitic diseases in the Amazon. *Acta Tropica* 129:33–41

de Thoisy B, Matheus S, Catzeflis F, Clément L, Barrioz S, Guidez A, et al. (2014) Maripa hantavirus in French Guiana: phylogenetic position and predicted spatial distribution of rodent hosts. *American Journal of Tropical Medecine Hygiene* 90:988–992

Lim BK (2012) Preliminary assessment of Neotropical mammal DNA barcodes: an underestimation of biodiversity. *Open Zoology Journal* 5:10–17

Matheus S, Djossou F, Moua D, Bourbigot AM, Hommel D, Lacoste V, et al. (2010) Hantavirus pulmonary syndrome, French Guiana. *Emerging Infectious Diseases* 16:739–741 . doi:10.3201/eid1604.090831

Meynard J-B, Flamand C, Dupuy C, Mahamat A, Eltges F, Queuche F, et al. (2012) First human rabies case in French Guiana, 2008: epidemiological investigation and control. *PLoS Neglected Tropical Diseases* 6(e1537):21 . doi:10.1371/journal.pntd.0001537

Milazzo ML, Cajimat MN, Haynie ML, Abbott KD, Bradley RD, Fulhorst CF (2008) Diversity among Tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the white-throated woodrat (*Neotoma albigula*) in the southwestern United States. *Vector-Borne Zoonotic Diseases* 8:523–554 . doi:10.1089/vbz.2007.0239

Nylander JAA (2004) *MrModeltest v2. Program Distributed by the Author*, Uppsala: Evolutionary Biology Centre, Uppsala University

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. (2012) MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61:539–542 . doi:10.1093/sysbio/sys029

Salvato MS, Clegg JCS, Buchmeier MJ, Charrel RN, Gonzales JP, Lukashevich IS, et al. (2005) V: Family Arenaviridae. In: *Virus Taxonomy. Eighth report of the International Committee on Taxonomy of Viruses*, Van Regenmortel MHV, Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (editors), San Diego (CA): Elsevier Academic Press, pp 725–738.

Wilson DE, Reeder DM (2005) *Mammal Species of the World. A Taxonomic and Geographic Reference*, Baltimore: The Johns Hopkins University Press

Woolhouse MEJ, Taylor LH, Haydon DT (2001) Population biology of multi-host pathogens. *Science* 292:1109–1112