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Short communication

Co-circulation of Clade C New World Arenaviruses: New geographic distribution and host species

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ABSTRACT

Clade C, of the New World Arenaviruses, is composed of only the Latino and Oliveros viruses and, besides the geographic range of their rodent reservoirs, the distribution of these viruses has been restricted to Bolivia and Argentina. In this study, the genetic detection and phylogenetic analysis of the complete S segment sequences of sympatric arenaviruses from Brazil revealed a new geographic distribution of clade C arenaviruses, as well as the association of Oliveros virus with a new rodent reservoir.

Arenaviruses are members of the family Arenaviridae that consists of two different genera (*Mammarenavirus* and *Reptarenavirus*) that currently comprises 30 viral species, as recognized by the International Committee for Taxonomy of Viruses (ICTV) (http://ictvdb.bio-mirror.cn/Ictv/fs_arena.htm [accessed 12 April 2015]). The geographic distribution of each arenavirus is assumed to be determined by the range of its reservoir species (Salazar-Bravo et al., 2002; Charrel and Lamballerie, 2010). The *Arenavirus* genus is phylogenetically and serologically divided into two main complexes: the LCMV–Lassa virus complex with all arenaviruses from the Old World, and the Tacaribe virus serocomplex that includes all viruses indigenous to the New World. The latter is the most genetically diverse group of the genus, composed of 18 species divided into four lineages: clade A, A-recombinant, B and C, according to their phylogenetic relationships (Charrel and Lamballerie, 2010; Charrel et al., 2008; Emonet et al., 2009).

Clade C New World Arenaviruses are the smallest lineage within the genus, with currently only two described members, the Latino (LAT) and Oliveros (OLV) viruses, hosted by *Calomys callosus* in Bolivia and *Necromys benefactus* in central Argentina, respectively (Rowe et al., 1970; Bowen et al.,1996a; Mills et al., 1996) (Fig. 1). In this study, we identified an expanded geographic distribution of these viruses, as well as the association of OLVV to a new rodent reservoir, *Necromys lasiurus*.

Small mammals trapping fieldwork was conducted in a rural area of Sidrolândia (20°55′55″S/54°57′39″W) and Dois Irmãos do Buriti (20°40′47″S/55°17′46″W) municipalities, in September 2005 and in January 2006, respectively. Those municipalities are situated in the Cerrado (savanna-like) area in Mato Grosso do Sul State of Brazil, where there is no description of arenavirus presence to date, although they are near regions where circulation of those viruses is well documented (Fig. 1). Each capture station was sampled with Sherman® (7.62 cm × 9.53 cm × 30.48 cm) and Tomahawk® (40.64 cm × 12.70 cm × 12.70 cm) live traps, placed 10 m apart, in linear ground transects of 20 capture stations, 270 traps per capture night. Small mammals were captured and handled according to recommended safety procedures (Mills et al., 1995). Seventy-two wild animals were captured (66 from Sidrolândia and six from Dois Irmãos do Buriti). *C. callosus* was the most frequently captured rodent (28 specimens), followed by *N. lasiurus* (21 specimens) (Table 1). The wild-caught animals were identified by external and cranial morphology and confirmed by karyotype and DNA analysis (Oliveira and Bonvicino, 2006;

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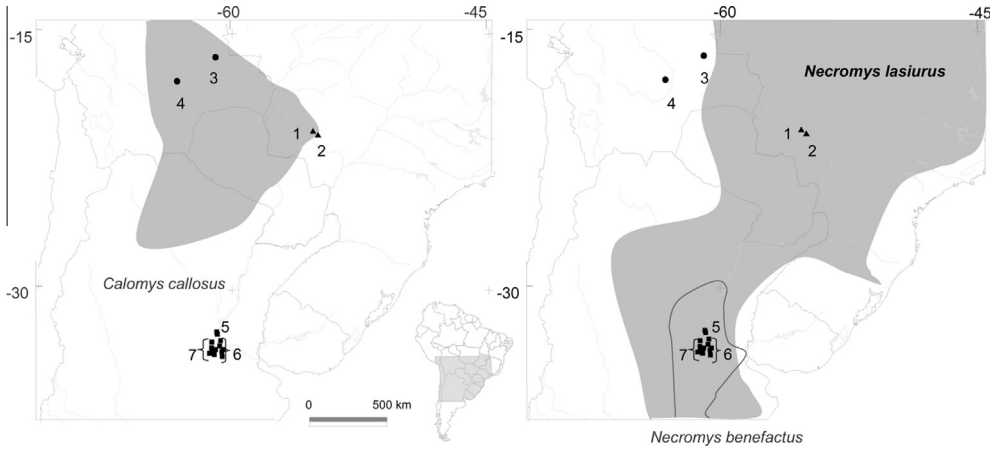

Fig. 1. Geographic distribution of Clade C New World Arenaviruses and their rodent hosts in South America. *Calomys callosus* (left map, dark gray area), *Necromys lasiurus*, *N. benefactus* (righ map). Square = Oliveros virus (OLVV) detection, circle = Latino virus (LATV) detection, triangle = co-circulation of OLVV and LATV. *Brazil*, *Mato Grosso do Sul*: (1) Dois Irmãos do Buriti, (2) Sidrolândia (present study). *Bolivia*, *Santa Cruz*: (3) San Ignacio, (4) Juan Latino (Cajimat et al., 2009). *Argentina*, *Buenos Aires*: (5) Oliveiros; *Santa Fé*: (5) Maciel, Pampa; (6) General Gelly, J.B. Molina, Maximo Paz, Pergamino, Uranga; (7) Alcorta, Bigand, Casilda, Chovet, Labordeboy, Wheelwright (Mills et al., 2007).

Table 1
Small mammal species collected on the two study sites and the results of arenavirus infection by RT-PCR in Mato Grosso do Sul State, Brazil.

Species	No. of captured rodents		RT-PCR no. (prevalence%)
	Sidrolândia	Dois Irmãos do Buriti	
Calomys callosus	27	1	19 (67.8%)[a]
Cerradomys maracajuensis	5	1	0
Hylaeamys megacephalus	2	0	0
Marmosa murina	2	0	0
Necromys lasiurus	21	0	3 (14.2%)[b]
Nectomys squamipes	3	0	0
Oecomys catharinae	0	2	0
Oligoryzomys sp.	2	0	0
Oxymycterus delator	2	0	0
Thrichomys fosteri	2	1	0
Thylamys macrurus	0	1	0
Total	66	6	21 (29.2%)

[a] Prevalence of Latino virus.
[b] Prevalence of Oliveros virus.

Bonvicino et al., 1996; Cassens et al., 2000). Tissue samples were collected and frozen immediately without any kind of medium. Sample specimens were deposited in the Rio de Janeiro National Museum.

Viral RNA was obtained using the PureLink Micro-to-Midi total RNA purification kit (Invitrogen, San Diego, CA) from frozen spleen or liver samples of all trapped animals. The amplification process was performed according to previously described protocols (García et al., 2000; Bowen et al., 1996b). Over 60% of *C. callosus* and 14% of *N. lasiurus* analyzed were infected by arenavirus in the municipalities of Sidrolândia and Dois Irmãos do Buriti (Table 1). High seroprevalence has been described previously for others arenavirus species in two other studies conducted in the Americas. One such example is a seroprevalence study of Tacaribe serocomplex arenaviruses in New Mexico, where 66% of *Neotoma albigula* were seroreactive (Cajimat et al., 2007). More recently, studies in endemic areas for Venezuelan haemorrhagic fever, demonstrated a seroprevalence of 64.9% of *Sigmodon alstoni* infected with Pirital virus (Milazzo et al., 2011).

For direct sequencing of overlapping amplimers, generic primer combinations were used for the amplification and sequencing of the complete genomic S segment, based on the conserved regions of the S segment among Clade C New World Arenaviruses (primers available on request) using a similar strategy to that described by Charrel et al. (2003). Complete sequence of LATV and OLVV S segment had approximately 3397 nt and 3545 nt, respectively.

Phylogenetic trees, based on the main proteins encoded by the arenavirus S segment, were generated by maximum likelihood method implemented with MEGA 6 (Tamura et al., 2013), under the best-fit model of evolution chosen by the program jModelTest (Posada, 2008). Partial and complete amino acid sequences from the Nucleoprotein (NP) and Glycoprotein precursor complex (GPC) (Fig. S1), and complete S segment nucleotide sequences (Fig. 2), formed a monophyletic clade, well supported by the New World Clade C *Arenavirus*. Viruses from *C. callosus* rodents were the closest related to LATV, while viruses from the *N. lasiurus* rodents were grouped with OLVV. The complete and partial amino acid sequences of the recovered proteins from *C. callosuś* virus showed greatest proximity, with a difference of 10.0% (NP) and 11.3% (GPC), to the LATV strain MARU (AF512830) obtained from rodent species *C. callosus* captured in Bolivia. Analysis of the similarity of NP and GPC proteins from viruses recovered from *N. lasiurus* showed the smallest differences, 11.2% and 16.4%, with OLVV (U34248) from central Argentina, harbored by the rodent *Necromys benefactus*.

As a result of the taxonomic complexity of the Arenaviridae family, some authors have suggested as a criterium for classification a cutoff value >12% of the uncorrected *p* distance of the nucleoprotein amino acid sequence along with other factors, such as, geographic distribution and natural reservoir (Charrel and Lamballerie, 2010; Emonet et al., 2009). Thus, despite the fact that the virus detected in *N. lasiurus* accomplishes two criteria for a new species: (i) has a distinct geographical distribution (ii) associated with a rodent species different from other arenaviruses reservoirs; the uncorrected *p*-distance of 11.2% of the nucleoprotein complete amino acid sequence, with its closest match, leads to the conclusion that the virus detected in *N. lasiurus* is a strain of OLVV. Additionally, a taxonomic revision of the genus *Necromys* spp. suggested that *N. benefactus*, OLVV reservoir in Argentina, is in fact a member of the *N. lasiurus* species (D'Élía et al., 2008).

The two arenaviruses identified in the present work do not match the taxonomic requirements for new species established by the ICTV. Thus, these viral strains were named as LATV Capão Seco strain and OLVV Quebra Coco strain. These names were given according to the political division of Sidrolândia municipality. The sympatric occurrence of Clade C New World Arenaviruses, even in

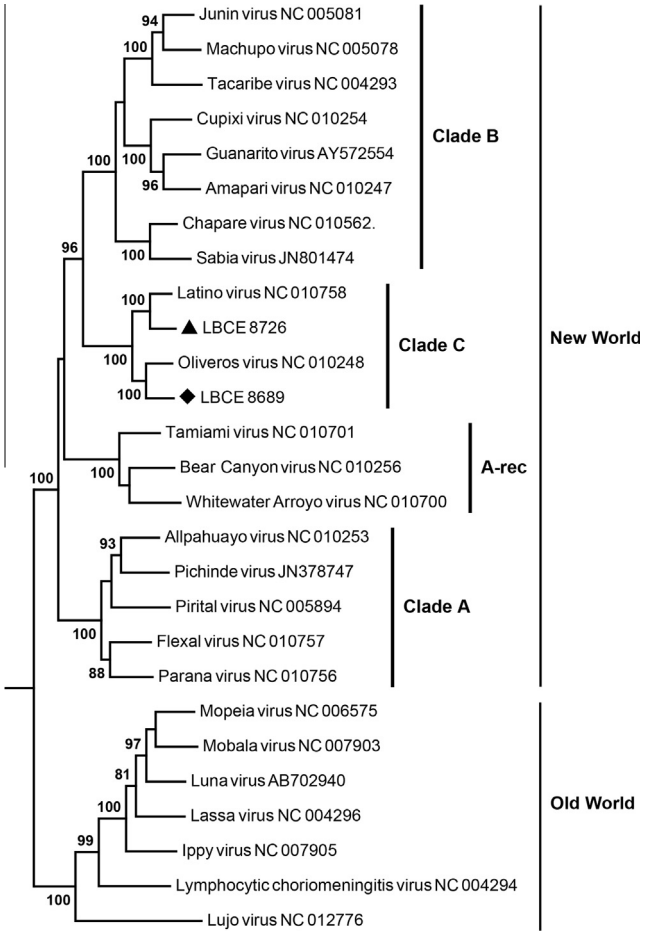

Fig. 2. Phylogenetic tree generated by maximum-likelihood, using the model HKY + G + I of evolution, based on the complete S segment nucleotide sequence. The numerical value >70% at the node indicates the Bootstrap, calculated from 1000 replicates. The scale bars indicate amino acid substitutions per site. Symbols represent virus species and geographic origins of the reservoir hosts: bold triangle = Latino virus Capão Seco strain obtained from *C. callosus*, Sidrolândia and bold rhombus = Oliveros virus Quebra Coco strain obtained from *N. lasisurus*, Sidrolândia.

the same trap station, as seen in this study, has not been reported before. This scenario suggests that these viruses have a very close relationship with their rodent hosts and do not seem to be shared among sympatric species. However, Mills et al. (2007) had found other rodent species with OLVV antibodies during a survey in central Argentina, a finding that has not yet been reported before for other New World Arenaviruses (Cajimat et al., 2007; Milazzo et al., 2011). This information, along with the fact that there are just a few ecological studies involving clade C arenaviruses, makes it clear that more studies need to be conducted to better understand the dynamics of these viruses and their hosts.

The presence of LATV in high prevalence, in the same reservoir species detected in Bolivia, reinforces the notion that most species of arenavirus are species-specific and that their occurrence is strictly related to the distribution of their reservoir, in this case the species *C. callosus*. Thus, it is possible to infer that probably the LATV distribution area includes dry and sub-humid regions of eastern Bolivia, northern Argentina, Paraguay and central-western Brazil, where the species *C. callosus* is present. A different aspect was found for OLVV, where the detection of this virus in a new reservoir corroborates the hypothesis proposed by Mills et al. (2007) that OLVV could be associated with the genus *Necromys* spp, of a wide geographic range, where many strains of this virus will probably be detected. This would be in agreement with the previously described OLVV strain Pampa virus (Lozano et al., 1997) also retrieved from a rodent genus *Necromys* spp. from Argentina, and the strains from this study.

The new distribution of Clade C New World Arenaviruses presented in this work demonstrates that these viruses are less geographically restricted than previously thought. These results highlight the importance of monitoring the occurrence and genetic diversity of South American arenaviruses and their rodent hosts to better understand their real territorial range.

Sequences accession number

Sequences generated during this work are deposited in GenBank database with the following accession numbers: (i) LATV Capão Seco strain partial GPC sequences: LBCE 8695 (KP027655), LBCE 8693 (KP027656), LBCE 8680 (KP027658), LBCE 8675 (KP027659), LBCE 8674 (KP027660), LBCE 8673 (KP027661), LBCE 8672 (KP027665), LBCE 8696 (KP027666), LBCE 8699 (KP027667), LBCE 8721 (KP027669), LBCE 8722 (KP027670), LBCE 8723 (KP027671), LBCE 8729 (KP027672), LBCE 8731 (KP027673), LBCE 8734 (KP027674), LBCE 8793 (KP027675); (ii) LATV Capão Seco strain partial NP sequences: LBCE 8673 (KM233886), LBCE 8675 (KP027662), LBCE 8674 (KP027663), LBCE 8672 (KP027664), LBCE 8693 (KM233887), LBCE 8695 (KM233888) LBCE 8699 (KM233889), LBCE 8721 (KM233890); (iii) LATV Capão Seco strain complete S segment sequence: LBCE 8726 (KP027676); (iv) OLVV Quebra Coco strain partial GPC sequences: LBCE 8684 (KP027657), LBCE 8710 (KP027668); (v) OLVV Quebra Coco strain complete S segment sequences: LBCE 8689 (KP027677).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2015.05.010.

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