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# First detection of adenovirus in the vampire bat (*Desmodus rotundus*) in Brazil

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**Abstract** This paper describes the first detection of adenovirus in a Brazilian *Desmodus rotundus* bat, the common vampire bat. As part of a continuous rabies surveillance program, three bat specimens were captured in Southern Brazil. Total DNA was extracted from pooled organs and submitted to a nested PCR designed to amplify a 280 bp long portion of the DNA polymerase gene of adenoviruses. One positive sample was subjected to nucleotide sequencing, confirming that this DNA fragment belongs to a member of the genus *Mastadenovirus*. This sequence is approximately 25 % divergent at the nucleotide level from equine adenovirus 1 and two other recently characterized bat adenoviruses.

**Keywords** *Desmodus rotundus* · Adenovirus · Rabies

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## Introduction

Short report

Bats (order *Chiroptera*) have increasingly been recognized as sources of viruses that can cause disease in humans and animals. Among these, a number of RNA viruses (lyssaviruses, coronaviruses, filoviruses, paramyxoviruses, and astroviruses) [1–4] as well as several DNA viruses (circoviruses [5], polyomaviruses [6], adenoviruses [7], and herpesviruses [8]) have been detected in bats, some of them with clear zoonotic potential, which highlights the importance of bat species as reservoirs for such agents.

As part of a larger study comprising the identification of novel bat viruses that could present zoonotic potential to humans and other mammals, this report concerns the identification of adenoviruses in tissues collected from the common vampire bat *Desmodus rotundus*. These are members of the *Adenoviridae* family, which to date comprises five known genera: *Mastadenovirus, Aviadenovirus, Atadenovirus, Siadenovirus,* and *Ichtadenovirus* (International Committee on Taxonomy of Viruses—9th report, 2011) [9]. The genus *Mastadenovirus* includes viruses that infect mammals and usually cause mild or asymptomatic infections. Nonetheless, some virus types/subtypes have often been associated with a wide range of clinical conditions, ranging from respiratory, ocular, and gastrointestinal disease in humans [10] to viral hepatitis and fatal respiratory infections in dogs [11].

In bats, the first adenovirus (AdV) reported was recovered from a fruit bat classified in the suborder *Megachiroptera,* during attempts to establish a specific cell line from the *Ryukyu flying fox* (*Pteropus dasymallus yaeyamae*) [12]. Later, the first AdV isolated from a microchiropteran bat (*Pipistrellus pipistrellus*) was reported in Germany and

considered a novel ADV by partial sequencing [13]. To date, two full genomes of bat adenoviruses have been published (Bat AdV1 and 2) [7, 14]. However, many partial and phylogenetically distant genes corresponding to the AdV DNA polymerase sequences have been deposited in GenBank. These *Mastadenovirus* sequences were from viruses detected in European and Asian bats of the genera *Hypsugo, Plecotus, Myotis, Nyctalus, Scotophilus, Pipistrellus, Hipposideros, Rousettus, Pteropus*, and *Rhinolophus*.

The michrochiropteran vampire bat (*Desmodus rotundus*) is widely distributed in Latin America and is a major reservoir for rabies virus [15]. Because rabies is a major concern in public health, most studies regarding *D. rotundus* have focused on their role in rabies epidemiology. A rather limited number of studies have been conducted to reveal the role of vampire bats as reservoirs for other viruses [16]. In light of the potential participation of these animals in the maintenance and spread of other viruses in nature, such as adenoviruses, this study was conducted in an attempt to identify AdV DNA in tissue samples of Brazilian bats. This is the first detection of adenoviruses in *D. rotundus*, and it contributes to the knowledge on the role of this species as a reservoir of viruses in nature.

Three specimens of the hematophagous "vampire" bat *D. rotundus* were captured in Rio Grande do Sul in Southern Brazil in 2012 under a controlled bat capture scheme that is part of a regional continuous rabies surveillance program. The bats were identified morphologically, and the identification was confirmed by amplification and sequencing of the mitochondrial cytochrome b (*cytb*) gene, as described previously [17]. The three bats were negative for rabies virus infection in routine tests performed by an official rabies diagnostic laboratory. Fragments of intestines, lungs, livers, and kidneys were pooled and subjected to total DNA extraction by standard phenol extraction [18].

The DNA samples were screened for the presence of AdV DNA by a nested PCR designed to amplify a 280 bp long fragment consisting of the partial DNA polymerase gene (*pol*), according to Li et al. [7]. Sequencing was performed with the Big Dye Terminator Cycle Sequencing Ready Reaction (Applied Biosystems, UK) in an ABI-PRISM 3100 Genetic Analyzer (ABI, Foster City, CA), following the manufacturer's protocol. Sequence analyses were performed with the BLAST software [19]. Nucleotide sequences were aligned and compared to partial DNA polymerase gene from bat AdVs sequences available at GenBank database with the program ClustalX 2.0 [20]. Alignments were optimized with the BioEdit Sequence Alignment Editor Program version 7.0.9 [21]. A maximum likelihood (ML) phylogenetic tree was built with the deduced amino acid sequence from *D. rotundus* N5.1 DNA polymerase and homologous adenoviruses sequences detected in different animal species deposited in GenBank. Bootstrap values were determined by 2000 replicates to assess the confidence level of each branch pattern. Phylogenetic analysis was carried out using MEGA5 [22].

**Table 1** Comparison of the deduced amino acid sequence identities based on the 280 bp partial fragment of DNA polymerase gene among Bat-AdV/POA/2012/N5.1 (KC110769) and other adenoviruses

| Bat AdV host | Accession No. | Country | DNA pol aa ID (%) |
|---|---|---|---|
| *Myotis ricketti* | ADD17139 | China | 79.3 |
| *Pipistrellus pipistrellus* | ACZ56364[a] | Germany | 79.1 |
| *Hypsugo savii* | AFO66610 | Spain | 77.8 |
| *Hypsugo savii* | AFO66611 | Spain | 77.8 |
| *Scotophilus kuhlii* | ADD17145 | China | 77.0 |
| *Myotis ricketti* | AFG28285 | China | 77.0 |
| *Myotis ricketti* | ADD17102[b] | China | 76.9 |
| *Nyctalus leisleri* | AFO66613 | Spain | 76.5 |
| *Nyctalus leisleri* | AFO66616 | Spain | 76.5 |
| *Myotis ricketti* | AFG28284 | China | 75.7 |
| *Plecotus austriacus* | AET11787 | Hungary | 75.3 |
| *Nyctalus lasiopterus* | AFO66615 | Spain | 74.1 |
| *Myotis horsfieldii* | ADD17127 | China | 73.9 |
| *Nyctalus lasiopterus* | AFO66609 | Spain | 72.8 |
| *Nyctalus lasiopterus* | AFO66612 | Spain | 72.8 |
| *Nyctalus lasiopterus* | AFO66614 | Spain | 72.8 |
| *Hipposideros armiger* | ADD17140 | China | 72.4 |
| *Nyctalus lasiopterus* | AFO66606 | Spain | 71.1 |
| *Rhinolophus ferrumequinum* | ADJ18279 | Hungary | 70.4 |
| *Pteropus dasymallus* | BAF93185 | Japan | 67.0 |
| *Myotis horsfieldii* | ADD17132 | China | 66.7 |
| *Rhinolophus ferrumequinum* | AET11786 | Hungary | 64.2 |
| *Rousettus leschenaultii* | AEO20260 | India | 61.5 |

[a] Bat AdV2 [14]

[b] Bat AdV1 [7]

One sample (N5.1) gave rise to an amplicon of the expected size (about 280 bp). This amplicon was cloned and sequenced three times, as described, and the sequence obtained was submitted to GenBank (Accession No. KC110769). A preliminary sequence analysis with BLAST confirmed the identity of N5.1 as a member of the genus *Mastadenovirus*. However, a comparison between the obtained sequence and

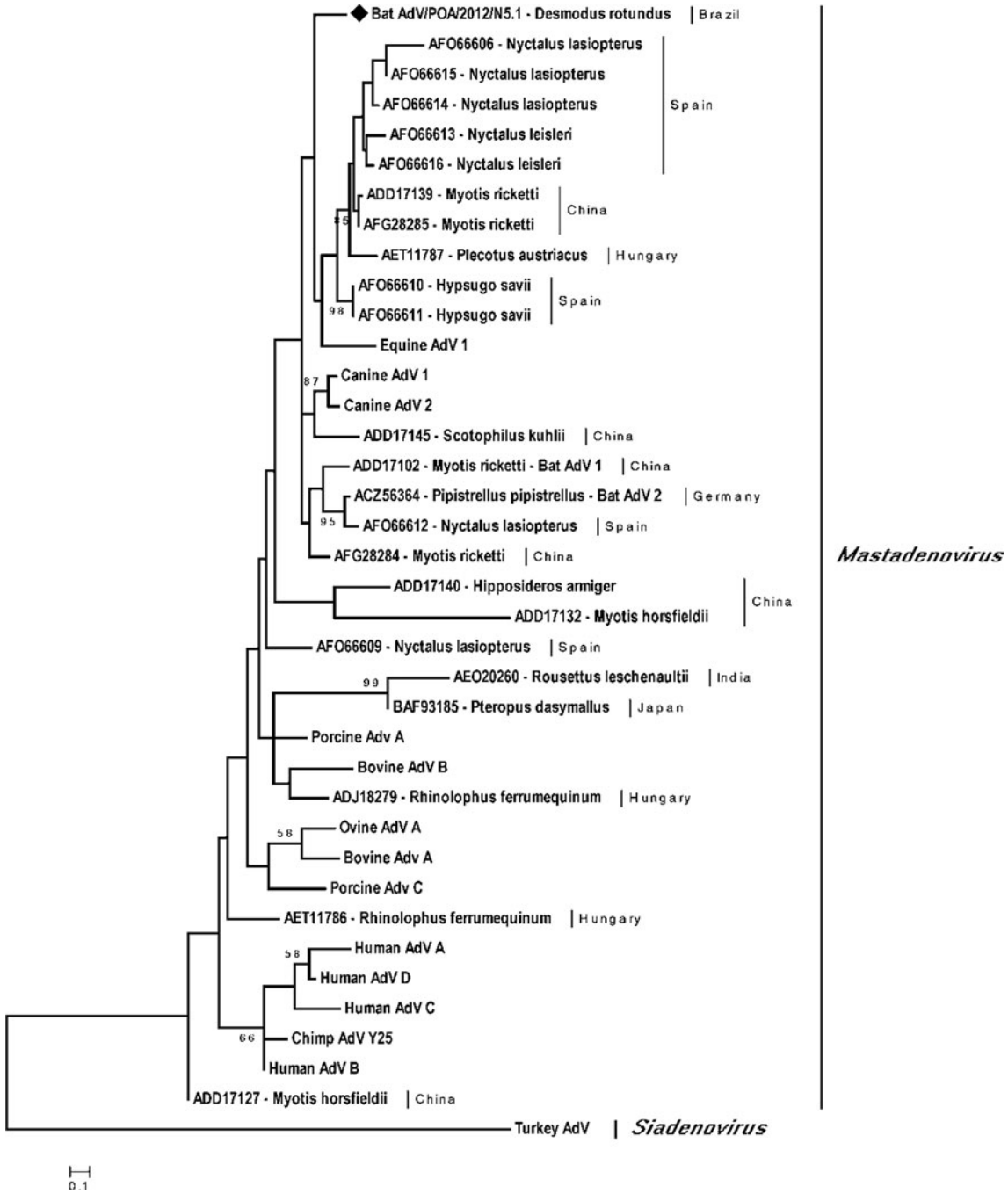


**Fig. 1** Molecular phylogenetic analysis by the ML method of adenoviruses based on an analysis of partial amino acid sequences of DNA polymerase protein from the 280 bp long sequence Bat AdV/POA/2012/N5.1 (KC 110769) and other *Mastadenoviruses*. The evolutionary history was inferred by using the maximum likelihood method based on the Poisson correction model. Evolutionary analyses were conducted in MEGA5 [22]

other bat AdVs sequences allowed us to conclude that N5.1 is genetically diverse from the bat adenoviruses already described (e.g., Bat AdV TJM and AdV2 PPV1), as they share nucleotide identities ranging from 66.2 to 72.7 % (supplemental material 1). Moreover, the amino acid identities between the sequence obtained in the present study and other bat AdVs ranged from 61.5 to 79.3 % (Table 1).

The phylogenetic analysis indicated that N5.1 forms a monophyletic clade with bat AdVs detected in Hungary [23], Spain (data unpublished), China [7], and Germany [24] (Fig. 1). These sequences, however, seem to be distinct from the homologous sequences of most AdVs detected in primates, some domesticated animals, humans, and some bat AdVs previously characterized in the Old World. The phylogenetic

tree topology suggests that coevolution is taking place involving most AdVs and bats species, as it is notable that different species host different adenoviruses [7].

A further comparison between N5.1 and other *Mastadenovirus* sequences shows that it is more closely related to AdVs of equine origin, such as *Equine adenovirus* 1 (EAdV-1), than to any other AdV sequence. N5.1 and EAdV-1 share nucleotide and amino acid identities of 74.5 and 75.8 %, respectively. This relationship is particularly noteworthy, as a previous genetic characterization of EAdV-1 revealed a close relationship with Bat AdV TJM and Bat AdV2 PPV1, suggesting that they may have a common ancestor [25]. On the other hand, previous deposited AdV sequences obtained from different bat species share higher identities to *Canine adenovirus* 1 and 2, which are known to display an unusually broad host range (e.g., bears, wolves, raccoons, and sea lions) [11, 13].

*Desmodus rotundus* is a serious threat to both animal and human health in Latin America, as it is a major reservoir for rabies virus in this subcontinent region [15]. This fact has limited investigations on the significance of bats as potential carriers of other viral pathogens. Nevertheless, intense epidemiological work is required to fully understand the genetic diversity and distribution of bat AdVs in Brazil. Such studies might be broadened and include other bat species considering the great variability of bats distributed in the country (about 140 species already identified) [15].

It must be highlighted that the amplified DNA polymerase gene fragment used in the phylogenetic analyses is short, allowing only a preliminary virus classification. More consistent sequence data from additional genomic regions will be required for an elucidative taxonomic classification of the N5.1 detected herein in *D. rotundus*. Further studies should be conducted in the future to examine the potential pathogenic role of bat AdVs to other species, providing new insights into the ecology and evolution of AdVs in different bat species.

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