Identification of Reptarenaviruses, Hartmaniviruses and a Novel Chuvirus in Captive Brazilian Native Boa Constrictors with Boid Inclusion Body Disease

Fernando Froner Argenta[1*], Jussi Hepojoki[2,3*,#], Teemu Smura[2], Leonora Szirovicza[2], Márcia Elisa Hammerschmitt[1], David Driemeier[1], Anja Kipar[3,4], Udo Hetzel[3,4]

The BIBD Group

[1]Department of Veterinary Pathology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

[2]University of Helsinki, Faculty of Medicine, Medicum, Department of Virology, Helsinki, Finland

[3]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

[4]University of Helsinki, Faculty of Veterinary Medicine, Department of Veterinary Biosciences, Helsinki, Finland

*** The authors contributed equally to this manuscript, the author order was determined alphabetically.**

Running title: Novel arenaviruses and a chuvirus in Brazilian boas

 # Corresponding author:

Present address*:* University of Helsinki
Medicum
Department of Virology
Haartmaninkatu 3
FI-00290 Helsinki
Finland
Phone: +358-50-4040243
E-mail: jussi.hepojoki@uzh.ch or jussi.hepojoki@helsinki.fi

Word number in abstract and importance: 221 and 143
Word number in text: 4687

ABSTRACT

Boid Inclusion Body Disease (BIBD) is a transmissible viral disease of captive snakes that causes severe losses in snake collections worldwide. It is caused by reptarenavirus infection, which can persist over several years without overt signs, but is generally associated with the eventual death of the affected snakes. Thus far, reports have confirmed existence of reptarenaviruses in captive snakes in North America, Europe, and Australia, but there is no evidence that it also occurs in wild snakes. BIBD affects both boas and pythons, the habitats of which do not naturally overlap. Herein, we studied Brazilian captive snakes with BIBD using a metatranscriptomic approach, and report the identification of novel reptarenaviruses, hartmaniviruses, and a new species in the family *Chuviridae*. The reptarenavirus L segments identified represent six novel species, while we only found a single novel reptarenavirus S segment. Until now, hartmaniviruses had been identified only in European captive boas with BIBD, and the present results increase the number of known hartmanivirus species from four to six. The newly identified chuvirus showed 38.4%, 40.9%, and 48.1% amino acid identity to the nucleoprotein, glycoprotein, and RNA-dependent RNA polymerase of its closest relative, Guangdong red-banded snake chuvirus-like virus. Although we cannot rule out the possibility that the found viruses originated from imported snakes, the results suggest that the viruses would circulate in indigenous snake populations.

IMPORTANCE

Boid Inclusion Body Disease (BIBD) caused by reptarenavirus infection affects captive snake populations worldwide, but the reservoir hosts of reptarenaviruses remain unknown. Herein, we report the identification of novel reptarenavirus and hartmanivirus species, and a chuvirus in captive Brazilian boas with BIBD. Three of the four snakes studied showed co-infection with all three viruses, and one of the snakes harbored three novel reptarenavirus L and one novel S segment. The samples originated from collections with Brazilian indigenous snakes only, which could indicate that these viruses circulate in wild snakes. The findings could further indicate that boid snakes are the natural reservoir of reptarena- and hartmaniviruses commonly found in captive snakes. The snakes infected with the novel chuvirus all suffered from BIBD; it is therefore not possible to comment on its potential pathogenicity and contribution to the observed changes in the present case material.

INTRODUCTION

The global decline in biodiversity is a topic of concern also for members of the class Reptilia. The worldwide transportation of wild caught, farm- and captive-bred reptiles facilitates also the transmission of pathogens. Thus, further information on reptilian pathogens is required to enable efficient screening of transported animals in order to secure e.g. zoological collections and to avoid spread of infectious agents into private and commercial breeding collections. Boid Inclusion Body Disease (BIBD), known to affect captive constrictor snakes, was recognized in the 1970s (1, 2), and arenaviruses were identified as the causative agent(s) in the early 2010s (3-10). BIBD affects nonvenomous constrictor snakes inhabiting biotopes in the neotropics and tropics. The natural habitats of boas include Central and South America, and Madagascar, while pythons are inherent in Africa, Asia and Australia. Although the habitats of boas and pythons do naturally not overlap geographically, snake species from several continents are housed together or in close proximity in zoological and private collections all around the world. As the name implies, BIBD manifests by the formation of eosinophilic and electron-dense inclusion bodies (IBs) within almost all cell types (2, 3, 5, 11). In fact, the ante mortem BIBD diagnosis relies on the detection of IBs in cytological specimens, e.g. blood smears (12, 13), or liver biopsies (1, 14). The identification of reptarenaviruses as the causative agent for BIBD has enabled RT-PCR based diagnostic procedures and screening of collections (12, 13, 15). Due to reasons unknown, BIBD is diagnosed more often in boas than in pythons (1, 10, 14). The disease can manifest itself with central nervous system (CNS) signs, which include opisthotonus (“star-gazing”), head tremors, disorientation, regurgitation and “corkscrewing” (1, 2). However, during the past decades, boas with BIBD and clinical CNS signs have become rare and even snakes with extensive IB formation often appear clinically healthy (10, 12, 14), which could be an indication of adaptation towards lower virulence. Instead, snakes with BIBD seem to emaciate

progressively and become terminally ill due to secondary, usually bacterial infections, presumably due to BIBD-associated immunosuppression (13).

In 2015, the BIBD associated arenaviruses were grouped to form the genus *Reptarenavirus* in the family *Arenaviridae*, and the formerly known arenaviruses of rodents and bats formed the genus *Mammarenavirus* (16). The mamm- and reptarenavirus genome is a bisegmented negative-sense RNA with ambisense coding strategy (17). The S segment encodes the glycoprotein precursor (GPC) and nucleoprotein (NP), and the L segment encodes the zinc finger matrix protein (ZP) and the RNA-dependent RNA polymerase (RdRp) (17). Co-incidentally, we identified Haartman Institute Snake virus-1 (HISV-1) in a snake with BIBD (9), and later demonstrated that the genome of HISV-1 is similar to that of mamm- and reptarenaviruses, except that it lacks the ZP gene (18). The identification of HISV-1 led to the formation of a third arenavirus genus, *Hartmanivirus* (19). The most recent addition to the family *Arenaviridae* is the genus *Antennavirus*, the representatives of which carry three instead of two genome segments (20). Others and we have demonstrated that snakes with BIBD often show co-infection with several reptarenavirus species (8, 9). We also identified further hartmaniviruses and showed that hartmaniviruses can co-infect snakes with BIBD (18). However, so far it is not clear whether hartmaniviruses contribute to BIBD pathogenesis.

The origin of reptarenaviruses and hartmaniviruses is still unknown, as reports have only described BIBD diagnosed in captive snakes. However, in order to gather information whether boid snakes themselves can be the viral reservoirs, it is of particular interest to see whether BIBD occurs within boid snake populations in the natural habitats. *Boa constrictors* are indigenous in Brazil, and the knowledge on reptarenavirus occurrence is limited to a single case report of a suspected BIBD case in *Corallus annulatus* kept in a zoological garden (21). In 2017, we diagnosed the first cases of BIBD in captive Brazilian *Boa constrictor* and undertook the present study to investigate the nature and phylogeny of the involved causative viruses.

RESULTS

Case descriptions

Clinical histories. Animals #1 and #4 died after unsuccessful therapeutic attempts (antibiotic and fluid therapy, catheter feeding) and chronic inflammatory processes in oral cavity and sinuses and a period of apathy, animal #3 died after a prolonged period of apathy and neurological signs, and animal #2 was found dead without prior clinical signs (Table 1).

Post mortem findings. At necropsy, animals #1-3 exhibited good body condition, whereas animal #4 was emaciated. All four snakes exhibited overt inflammatory processes: a chronic ulcerative stomatitis and osteomyelitis of the maxilla (animals #1 and #3) (Fig. 1), a multifocal ulcerative deep dermatitis and myositis extending to the vertebral bones (animal #1), a fibrino-necrotizing cloacitis (animal #2), and a chronic suppurative sinusitis (animal #4). Histological examination confirmed the findings. In animal #1, the stomatitis was predominantly heterophilic (i.e. suppurative); bacteriology and mycology isolated *Enterobacter gergoviae*, *Providencia ssp*., *Proteus ssp*., and *Candida albicans*, respectively, from the lesions. The inflammatory infiltrate of the cloacitis in animal #2 was heterophil-dominated, with abundant aggregates of coccoid bacilli within the superficial layer of fibrin and necrotic debris. This animal exhibited multifocal areas of necrosis with embedded aggregates of coccoid bacilli in the kidneys, consistent with embolic-metastatic nephritis as a consequence of bacteremia. A bacteriological examination was not performed. In animal #3, the stomatitis was granulomatous, with multinucleated giant cells; only non-specific flora (*Klebsiella ssp*.) was isolated from the lesion. In the sinusitis of animal #4 heterophils were the predominant inflammatory cells; the bacteriological examination of a sample from this lesion yielded exuberant mixed growth (*Enterobacter gergoviae* and *Pseudomonas* ssp.). In addition, this animal exhibited a mild multifocal heterophil-dominated pneumonia.

In all snakes, histology served to confirm BIBD. The characteristic eosinophilic intracytoplasmic IBs were found in parenchymal cells in a range of organs (brain (Figs. 2A) and spinal cord, liver (Fig. 3A), pancreas, lungs, kidneys) in all animals. The IBs varied in size distribution, indicating a chronic stage of the disease (Figs. 2A and 3A).

Identification of reptarenaviruses, hartmaniviruses, and a chuvirus.

To identify the infecting viruses, we isolated RNA from liver samples and performed a metatranscriptomic analysis, an approach we have successfully applied in earlier studies (9, 18, 22-24). We used the Basic Local Alignment Search Tool (BLAST, at https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the viral sequences. The sequencing confirmed all snakes to be reptarenavirus infected, and similarly to earlier observations (8, 9, 22), all snakes harbored several L segments; however, we identified only a single S segment in each snake (Table 2). In addition to reptarenaviruses, the analysis revealed the presence of one hartmanivirus S and L segment pair in three of the four snakes (animals #1-3) studied (Table 2). We recovered complete coding sequences (CDSs) for one L and two S segments, and nearly complete CDS (covering >95% of the segment) of an additional hartmanivirus L segment. Genome *de novo* assembly using the sequence data obtained from these three snakes also produced close to identical contigs, varying in length from 10549 to 10718 nt (Table 2), that showed highest matches in the BLAST analysis to chuvirus-like viruses. Table 2 contains the virus names, contig lengths, GenBank accession numbers, and average coverages for the viruses identified, and Figure 4 shows the contig coverages nucleotide-by-nucleotide.

Immunohistology served to detect reptarenavirus and hartmanivirus NP in cells with IB. Reptarenavirus NP expression was mainly restricted to the IBs (Figs. 2B and 3B, C), whereas hartmanivirus NP was also detected throughout the cytoplasm (Fig. 2C).

Analysis of the identified reptarenavirus sequences.

We used the PAirwise Sequence Comparison (PASC) web tool (available at https://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage=overview), recommended by the *Arenaviridae* study group of the International Committee on Taxonomy of Viruses (ICTV) for arenavirus classification (16), to analyze the identified reptarenavirus segments. The PASC results showed that we had recovered CDSs for seven novel L and two novel S segments (Table 2). The PASC analysis identified one of the L segments in snake #1 as UHV-

2 (86.4% nucleotide, nt, identity), whereas in BLAST analysis three L segments in snakes #1-3 were >97% identical to Kaltenbach virus-1 (KaBV-1) (22), which is apparently not included in the PASC reference data set. Six L segments had less than 76% nucleotide identity to any currently known reptarenavirus sequences; Table 3 shows the nucleotide identity matrixes of the reptarenavirus segments. The analyses confirmed that we had recovered L segment CDSs for six novel reptarenavirus species (Tables 2 and 3): Aramboia boa virus-1 (ArBV-1, in animals #1-3), Arabuta Snake virus-1 (ArSV-1, in animal #1), Frankfurter Strasse virus-1 (FStV-1, in animal #1), Porto Alegre virus-1 (PAV-1, in animal #4), Saudades virus-1 (SauV-1, in animal #4), and Gaucho virus-1 (GauV-1, in animal #4). We found only a single S segment CDS for each of the studied snakes, and chose to name the S segments according to the L segment with highest coverage found in the same snake (Table 2): ArBV-1 (in animals #1-3) and PAV-1 (in animal #4). Phylogenetic analysis of the reptarenavirus L (Fig. 5) and S segments (Fig. 6A and B) showed the viruses to be distant from those present in GenBank, supporting their assignment as novel species. Notably, the reptarenavirus sequences recovered from the Brazilian snakes did not show geographical clustering but interspersed among the virus species detected from captive snakes in Europe and USA. As discussed previously (8), the phylogenetic trees constructed based on NP and GPC coding regions of reptarenavirus had incongruent topologies, suggesting recombination events to have occurred during the evolution of reptarenavirus S segments.

Analysis of the identified hartmanivirus sequences.

We used the PASC tool also for analyzing the identified hartmanivirus S and L segment CDS, however, the analyses returned matches with very low sequence identities (22% and below, Table 2). To compare the sequences to known hartmaniviruses, we aligned the identified sequences with those found in the GenBank and generated nucleotide identity matrixes (Table 4). The analysis showed that the sequences are distant enough from each

other and the known hartmaniviruses to represent new species: SetVetPat virus-1 (SPVV-1, in animal #1) and Andere Heimat virus-1 (AHeV-1, in animals #2 and #3). The phylogenetic analysis of hartmanivirus L and S segments suggested that these two viruses form a sister clade to the previously known hartmaniviruses (Fig. 7A-C).

Analysis of the novel species in the family *Chuviridae*.

BLAST analysis identified three contigs that showed similarities to chuvirus-like viruses (family *Chuviridae*, genus *Mivirus*). These sequences had three ORFs in antigenomic orientation, representing the L, G, and N gene with RNA-dependent RNA polymerase (RdRp), glycoprotein (GP), and nucleoprotein (NP) as the respective protein products (Fig. 8A). We named the novel virus as Herr Frank virus-1 (HFrV-1, GenBank accession numbers in Table 2). BLAST analysis identified Guangdong red-banded snake chuvirus-like virus L protein (GenBank accession no. AVM87272.1) as the closest match (48.56% amino acid identity) for the HFrV-1 L gene (Fig. 8B)and putative GP (AVM87273.1) and NP (AVM87274.1) of as the closest matches for HFrV-1 GP and N gene (respective amino acid identities of 43.06% and 41.97%), Fig. 8B. In addition to BLAST analysis, we employed HMMSCAN (available at https://www.ebi.ac.uk/Tools/hmmer/search/hmmscan) to study the ORFs of HFrV-1. The analyses, presented in Fig. 8B, further confirmed the annotation of ORFs. The phylogenetic analysis of HFrV and the representatives of other chu-like virus RdRp sequences suggested that HFrV clusters together with Guangdong red-banded snake chuvirus-like virus and Wenling fish chu-like virus (25). Sanxia atyid shrip virus 4 formed an outgroup for these three vertebrate-associated chu-like viruses.

DISCUSSION

This study aimed to confirm the presence of BIBD and reptarenaviruses in *Boa constrictors*, indigenous to the Brazilian wildlife. We initially used immunohistology to confirm the presence of reptarenavirus-induced IBs in all four studied snakes, thus confirming the BIBD diagnosis. The subsequent metatranscriptomic analysis of the livers confirmed the presence of reptarenaviruses, and helped to identify two novel hartmanivirus species and a novel chuvirus in the snakes. The affected boas had lived in captivity, with contact to other snakes. However, three of the snakes originated from the Amazon region in Brazil, which could indicate that both reptarenaviruses and hartmaniviruses exist in wild snakes in this region.

At the time of sampling/euthanasia, all snakes suffered from bacterial (and fungal) infections, caused by opportunistic agents that are part of the mucosal flora in various species including snakes. These agents occur in the oral cavities of captive snakes with stomatitis or in snakes with septicemia (26, 27); some, like *Enterobacter gergoviae*, exist in water or soil (28). All can cause disease, particularly in immunocompromised patients. Secondary infections with related inflammatory processes are common in snakes with BIBD, indicating an immunosuppressive effect at least in later, chronic stages of BIBD. Much less is known about the effect of acute reptarenavirus infections. Based on our experience with *in vitro* studies, we assume that larger, often more amphophilic than eosinophilic IBs represent late stages of IB formation. Such IBs often exhibit only a peripheral positive immunohistological reaction for viral NP, similarly to the reaction seen in the tissues of the snakes in this study. *In vitro*, small irregular IBs appear at early stages (approx. 3 days post infection, dpi) of reptarenavirus infection, and from 12 dpi onwards the IBs become larger and more electron dense (unpublished data). Thus, we assume that the snakes included in this study were chronically reptarenavirus infected, and therefore immunocompromised (the hartmanivirus

211 and a chuvirus co-infections may also have contributed), which in turn led to the observed
212 secondary infections.

213 Metatranscriptomic analysis of the animals revealed the presence of multiple
214 reptarenavirus L segments, but only a single S segment per snake. The finding is very
215 surprising, since others and we have observed reptarenavirus co-infections to be common in
216 snakes with BIBD (8, 9). One of the identified segments, the KaBV-1 L segment (animals #1-
217 3), showed a striking 97% identity to a previously identified (22) reptarenavirus genome
218 segment. In addition, one L segment showed approximately 86% identity to the UHV-2 L
219 segment, but the other segments differed enough from the previously identified reptarenavirus
220 segments to warrant classification as novel reptarenavirus species. Interestingly, we did not
221 find University of Giessen virus or "S6-like" S segment in any of the studied snakes, even
222 though the segment is most often reported in captive snakes with BIBD (8, 9, 13, 22). In the
223 phylogenetic trees, most of the reptarenaviruses identified from the Brazilian snakes
224 interspersed among the virus species previously detected from captive snakes in Europe and
225 USA. Notably, one of the reptarenavirus species, KaBV-1, showed high identity to a strain
226 identified from captive snakes in Switzerland (22). This may indicate that reptarenaviruses
227 have been introduced to captive snake populations by wild boas and then been exported with
228 them, due to the lack of clinical signs of infection/disease (13). A great number of snakes are
229 traded annually, according to CITES 20,000 snakes (6,600 pythonids and 3,100 boids) were
230 transported in 2018 alone (https://trade.cites.org/en/cites_trade/#). While the trading and
231 transport may technically follow CITES regulations, a great number of transported animals
232 are likely not captive bred but wild-caught. In addition to the transport of animals following
233 CITES regulations, smuggling of wild-caught snakes occurs frequently. The spread would
234 further be aided by the fact that reptarenavirus infection does not induce clinical signs rapidly,
235 especially not in boas (10), and is vertically transmitted (22). The studies on snakes with
236 BIBD strongly suggest that reptarenavirus L and S segments are able to pair with each other

237 rather freely, since most often the individuals harbor more L than S segments (8, 9, 22).
238 Assuming that snakes, or better boas and pythons, are the reservoir hosts of reptarenaviruses
239 and that reptarenaviruses have co-evolved with their reservoir hosts, then multiple cross-
240 species transmission events could explain the status quo in captive snakes. However, with the
241 current set of data we cannot rule out the possibility that the wild-caught boas included in the
242 study had not been infected during co-housing.

243 The identification of novel hartmanivirus species in Brazilian *B. constrictor* snakes is
244 interesting, since up to now hartmanivirus infection has only been reported in European
245 captive snakes (9, 18). The hartmanivirus infected snakes included in the present study had
246 developed BIBD as confirmed by the presence of IBs in both blood smear and tissues, which
247 is in accordance with our earlier findings (9, 18). They showed strong expression of
248 hartmanivirus NP in parenchymal cells in various organs. We have thus far detected
249 hartmaniviruses mainly in snakes with BIBD, however, the fact that we mainly look for
250 viruses in diagnostic cases might introduce a bias and could explain the seeming correlation
251 between hartmanivirus infection and BIBD. In fact, when studying samples collected from a
252 single breeding colony for the presence of IBs, reptarenaviruses, and hartmaniviruses, we did
253 not find a significant correlation between hartmanivirus infection and BIBD (13). Although
254 hartmanivirus infection appears to most often occur simultaneously to reptarenavirus
255 infection, hartmaniviruses do can infect and replicate without a co-infecting reptarenavirus
256 (18) and further studies need to address their pathogenicity. Alike reptarenaviruses, the origin
257 of hartmaniviruses remains unknown. In addition to snakes being the reservoir hosts of the
258 viruses, one could speculate that blood-feeding parasites e.g. mites, ticks, mosquitoes, etc.
259 would serve as reservoirs and/or vectors in virus transmission.

260 The novel chuvirus, HFrV-1, found in three BIBD positive snakes originating from a snake
261 sanctuary in the Amazonas region, but housed in a smaller colony for several years
262 afterwards, was an unexpected finding. By amino acid identity, the closest relative to the

newly found mononegavirus is the Guandong red-banded snake Chuvirus, which was identified from a liver sample of a Chinese snake (25). In general, the identification of chu-like viruses in fish and snakes from different continents (Asia and the Americas) suggests that chuviruses might be common and geographically widespread. Due to bacterial and viral co-infections in the snakes with HFrV-1 infection, we cannot draw conclusions on the potential morbidity of HFrV-1. The fact that the identified viruses showed nearly identical sequences suggests that the Chuvirus infection may have occurred during captivity.

According to Whitfield et al, the major threats of declining reptilian populations are habitat loss and degradation, introduction of invasive species, environmental pollution, disease, unsustainable use and global climate change (29). Our results raise an obvious question: From where did the reptarenaviruses come that infected the diseased snakes? Both co-housed imported snakes and local wild snakes are a potential source of infection. Animals #1-3 originated from the Amazonas region, where they resided in a snake sanctuary before moving to a private collection in Porto Alegre. It was not possible to obtain information on other snake species housed in the sanctuary, since it was closed several years back. We also lack more specific information on the origin of the snakes. Similarly, the origin of animal #4 remained unknown. However, all animals studied were *B. constrictor*, indigenous to Brazil, and it is therefore possible that they originated from the wild. If snakes are not the reservoir hosts of reptarenaviruses, then the occurrence of BIBD-positive *B. constrictor* in Brazil is an alarming signal posing a potential threat to Brazilian wild *B. constrictor* populations.

MATERIALS AND METHODS

Animals. The study was undertaken on four captive adult *B. constrictor constrictor* snakes. Three derived from a single private owner (animals #1-3), the fourth from a zoological garden (Table 1). All animals were submitted for diagnostic post mortem examination to the Department of Veterinary Pathology in Porto Alegre. Tissue specimens were fixed in 10% buffered formalin for histological and immunohistological examination. Additional sets of samples were stored frozen in RNAlater™ Stabilization Solution (ThermoFischer Scientific) for RNA extraction. For animals #1, #3 and #4, samples from the oral and nasal lesions, respectively, were subjected to a routine bacteriological examination; for animal #1, a routine mycological examination was also performed.

Histology and immunohistology. Formalin-fixed tissue specimens were trimmed and routinely paraffin wax embedded. Consecutive sections (3-5 µm) were prepared and stained with hematoxylin-eosin (HE) and special stains (Periodic acid-Schiff (PAS) reaction, Grocott methenamine silver stain), when appropriate. Further sections were subjected to immunohistological staining for reptarenavirus and hartmanivirus NP as described (5).

Antibodies, protein expression, and immunization. The anti-UHV NP and anti-UHV NP C-terminus antibodies were described earlier (5, 30). To generate broadly cross-reactive antiserum against reptarenavirus NPs, we performed amino acid alignment for the reptarenavirus NP available in GenBank. Based on homology between the sequences, we selected the following regions: amino acids 47-140 from UGV-1 (GenBank: YP_009508464.1), 173-224 from UHV-1 (YP_009019205.1), 233-270 from UHV-1 (YP_009019205.1), 286-359 from UGV-1 (YP_009508464.1), 208-280 from UGV-1 (YP_009508464.1), and 494-567 from UHV-1 (YP_009019205.1). To generate a cross-reactive antiserum against hartmanivirus NPs, we used the same approach and selected the following regions: amino acids 199-256 from Veterinary Pathology Zürich virus-1, VPZV-1 (AZI72586.1); 132-180 from Haartman Institute Snake virus-2, HISV-2 (AZI72594.1); 257-

299 from VPZV-1 (AZI72586.1), and 312-364 from VPZV-2 (AZI72596.1). We included five glycine residues between the selected epitopes, and ordered the engineered proteins as synthetic genes optimized for *E. coli* expression in pET-20b(+) plasmid from GenScript. We transformed One Shot™ BL21(DE3) Chemically Competent (Thermo Scientific) *E. coli* with the plasmids following the manufacturer's protocol, and performed protein expression and purification via his-tag as described (23, 30). Antisera against the purified proteins were raised by BioGenes, as described in earlier studies (23, 24, 30). We designated the novel antisera as anti-pan-RAV and anti-pan-hartmani.

Next generation sequencing (NGS) and genome assembly. We extracted RNAs for NGS from liver samples stored frozen in RNAlater™ (the sample from animal #1 had been kept at ambient temperature for a few weeks prior to extraction) as described (22), prepared NGS libraries, and performed sequencing and subsequent genome assembly as described (22, 23).

Reverse transcriptase-polymerase chain reaction (RT-PCR) and Sanger sequencing. For the sample that had been stored in RNAlater at ambient temperature (animal #1) we obtained open reading frames (ORFs) for several NP, ZP, GPC, and RdRp genes instead of complete L and S segments by NGS and de novo assembly. To complete the L and S segment sequences, we designed the following primers to amplify the missing intergenic regions: Br_GPC1 5´-ACACTTGGATTCTATGGGAGT-3´, Br_NP1 5´-ACTGCATGGTGTTCTCAAG-3´, Br_ZP1 5´-GAGTCTAACCAATCCCAGAA-3´, Br_ZP2 5´-CATGCCTAATGGCAAAAC-3´, Br_ZP3 5´-CAGAATGTAGGGCAACAC-3´, Br_ZP4 5´-AGGGTCTAAATCAACATCCC-3´, Br_UHV_RdRp 5´-GTCAGAATATCACTCCTGGAG-3´, Br_RdRp2 5´-TAGGGTGACACTTTTGAAGG-3´, Br_RdRp3 5´-GAACATTAGGGTATCACTCCTC-3´, and Br_RdRp4 5´-AGAGTCTAAGGGTCCTGGA-3´. We performed RT-PCR with all primer combinations (ZPs with RdRps, and GPC with NP) as described in (22), and used GeneJET Gel Extraction Kit (ThermoFisher Scientific) to purify the RT-PCR products which were further cleaned by

AMPure XP beads (Beckman Coulter) before ligation to plasmid using the Zero Blunt TOPO PCR Cloning Kit (ThermoFisher Scientific); all steps were according to the manufacturer's instructions. Chemically competent *E. coli* (TOP10, ThermoFisher Scientific) transformed with the ligated plasmids were grown on LB plates with 100 μg/ml of ampicillin O/N at 37 °C, colonies were picked and grown in 5 ml of 2x YT medium with 100 μg/ml of ampicillin O/N at 37 °C. The plasmids were purified from 2 ml of the O/N culture using the GeneJET Plasmid Miniprep Kit (ThermoFisher Scientific), and sent for Sanger sequencing to DNA Sequencing and Genomics, Institute of Biotechnology, University of Helsinki.

Phylogenetic analysis. The amino acid sequences of the representatives of all reptarena- and hartmanivirus species were downloaded from the GenBank, and aligned with the amino acid sequences of viruses identified in this study using the MAFFT E-INS-i algorithm (31). For chuvirus-like sequences, 100 closest BLASTx matches for the putative RdRp amino acid sequence were downloaded from GenBank and aligned as indicated above. Redundant sequences (fragmental and identical sequences) were removed from the dataset.

The best-fit amino acid substitution models and phylogenetic trees were inferred using the Bayesian Monte Carlo Markov Chain (MCMC) method implemented in MrBayes v3.2.6. (32). MrBayes was run for 500,000 generations and sampled every 5,000 steps, with final standard deviations between two runs < 0.02 for all analyses. The analyses were carried out at the CSC server (IT Center for Science Ltd., Espoo, Finland).

Data availability. The names for newly sequenced viruses with corresponding abbreviations and GenBank accession numbers are provided in Table 2.

FIGURE LEGENDS

Figure 1. Oral cavity of snakes with confirmed BIBD. **A**. Animal #1. Chronic ulcerative stomatitis. L: larynx. **B**. Animal #3. Focally ulcerated chronic stomatitis. Ch: choana.

Figure 2. Histological and immunohistological findings in the brain of animal #3. **A**. Neurons exhibit the typical cytoplasmic eosinophilic IBs (arrowheads) which vary in size and can reach the size of and obscuring the nucleus (arrows). HE stain. **B**. Staining with the anti-pan-RAV antibody highlights the IBs depicted in the HE stain. Immunohistology, hemalaun counterstain. **C**, Staining with the anti-pan-hartmani antibody highlights the IBs, but also shows the presence of NP within the entire cytoplasm of infected cells (arrow and inset). Immunohistology, hemalaun counterstain.

Figure 3. Histological and immunohistological findings in the liver of animal #4. **A**. Numerous hepatocytes exhibit a cytoplasmic eosinophilic IB of variable size (arrowheads). HE stain. **B**. Staining with the anti-UHV NP antibody highlights individual IBs (arrowheads) and shows that some cells contain several small IBs (short arrow). Some larger IBs appear negative (large arrows). Immunohistology, hemalaun counterstain. **C.** Staining with the anti-pan-RAV antibody shows the presence of abundant individual (arrowheads) and multiple IBs (short arrows) within hepatocytes. Again, a few larger IBs appear negative (large arrow). Immunohistology, hemalaun counterstain.

Figure 4. Coverages of the genomes and genome segments assembled. For all graphs: the y-axis represents the coverage and the x-axis indicates the nucleotide position.

Figure 5. Maximum clade credibility tree of reptarenavirus L segments. The tree was constructed from amino acid sequences of the representatives of all reptarenavirus species and the strains identified in this study, using Bayesian MCMC method with Jones model of amino acid substitution. Posterior probabilities are shown in each node.

Figure 6. Maximum clade credibility trees of reptarenavirus GPCs and NPs. **A)** The phylogenetic tree based on the GPC amino acid sequences of the viruses identified in this study and those available in GenBank was constructed using Bayesian MCMC method with Blosum model of amino acid substitution. **B)** The phylogenetic tree based on the NP amino acid sequences of the viruses identified in this study and those available in GenBank was constructed using Bayesian MCMC method with Jones model of amino acid substitution.

Figure 7. Maximum clade credibility trees for hartmanivirus RdRp, GPC, and NP. **A)** The phylogenetic tree based on the RdRp amino acid sequences of the viruses identified in this study and those available in GenBank was constructed using Bayesian MCMC method with Blosum model of amino acid substitution. **B)** The phylogenetic tree based on the GPC amino acid sequences of the viruses identified in this study and those available in GenBank was constructed using Bayesian MCMC method with Blosum model of amino acid substitution. **C)** The phylogenetic tree based on the NP amino acid sequences of the viruses identified in this study and those available in GenBank was constructed using Bayesian MCMC method with Wag model of amino acid substitution.

Figure 8. Genome organization, similarity analyses, and phylogenetic tree of HFrV-1. **A)** Genome organization and coverage (sequence from snake #1) of HFrV-1. The arrows represent the orientation of the open reading frames (ORFs). The L gene encodes RNA-dependent RNA polymerase (RdRp), G gene encodes glycoprotein (GP), and the N gene encodes nucleoprotein (NP). The coverage (y-axis) show sequencing depth at each nucleotide position (x-axis). **B)** Primary sequence, BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and HMMSCAN (https://www.ebi.ac.uk/Tools/hmmer/) analysis of the HFrV-1 ORFs. **C)** A maximum clade credibility tree based on the RdRp amino acid sequences of chu-like and chuviruses. The tree was constructed using Bayesian MCMC method with Blosum model of amino acid substitution. Posterior probabilities are shown in each node.

406 **ACKNOWLEDGEMENTS**

407 The authors are grateful to the laboratory staff of the Histology Laboratory, Institute of
408 Veterinary Pathology, Vetsuisse Faculty, University of Zürich, and colleagues and professors
409 of Universidade Federal do Rio Grande do Sul for excellent technical support. They also wish
410 to thank L Sonne and SP Pavarini who assisted in the snake necropsies during their
411 undergraduate studies. The study received financial support from the Leading House for the
412 Latin American Region, University of St. Gallen, Switzerland, and the Academy of Finland
413 (grant numbers 1308613 and 1314119). This work is dedicated to the memory of Dr. Herbert
414 Frank and to his colleagues at the Institute of Veterinary Pathology, Faculty of Veterinary
415 Medicine, Justus-Liebig-University Giessen, Germany, where the three senior authors of this
416 publication were trained in veterinary pathology.

417

418

419

REFERENCES

1. **Chang, L., and E. R. Jacobson.** 2010. Inclusion Body Disease, A Worldwide Infectious Disease of Boid Snakes: A Review. Journal of Exotic Pet Medicine. **19:**216-225. doi: 10.1053/j.jepm.2010.07.014.

2. **Schumacher, J., E. R. Jacobson, B. L. Homer, and J. M. Gaskin.** 1994. Inclusion Body Disease in Boid Snakes. Journal of Zoo and Wildlife Medicine. **25:**pp. 511-524.

3. **Stenglein, M. D., C. Sanders, A. L. Kistler, J. G. Ruby, J. Y. Franco, D. R. Reavill, F. Dunker, and J. L. Derisi.** 2012. Identification, characterization, and in vitro culture of highly divergent arenaviruses from boa constrictors and annulated tree boas: candidate etiological agents for snake inclusion body disease. MBio. **3:**e00180-12. doi: 10.1128/mBio.00180-12; 10.1128/mBio.00180-12.

4. **Bodewes, R., M. J. Kik, V. S. Raj, C. M. Schapendonk, B. L. Haagmans, S. L. Smits, and A. D. Osterhaus.** 2013. Detection of novel divergent arenaviruses in boid snakes with inclusion body disease in The Netherlands. J. Gen. Virol. **94:**1206-1210. doi: 10.1099/vir.0.051995-0; 10.1099/vir.0.051995-0.

5. **Hetzel, U., T. Sironen, P. Laurinmaki, L. Liljeroos, A. Patjas, H. Henttonen, A. Vaheri, A. Artelt, A. Kipar, S. J. Butcher, O. Vapalahti, and J. Hepojoki.** 2013. Isolation, identification, and characterization of novel arenaviruses, the etiological agents of boid inclusion body disease. J. Virol. **87:**10918-10935. doi: 10.1128/JVI.01123-13; 10.1128/JVI.01123-13.

6. **Bodewes, R., V. S. Raj, M. J. Kik, C. M. Schapendonk, B. L. Haagmans, S. L. Smits, and A. D. Osterhaus.** 2014. Updated phylogenetic analysis of arenaviruses detected in boid snakes. J. Virol. **88:**1399-1400. doi: 10.1128/JVI.02753-13; 10.1128/JVI.02753-13.

7. **Hetzel, U., T. Sironen, P. Laurinmaki, L. Liljeroos, A. Patjas, H. Henttonen, A. Vaheri, A. Artelt, A. Kipar, S. J. Butcher, O. Vapalahti, and J. Hepojoki.** 2014. Reply to

"updated phylogenetic analysis of arenaviruses detected in boid snakes". J. Virol. **88:**1401-13. doi: 10.1128/JVI.03044-13; 10.1128/JVI.03044-13.

8. **Stenglein, M. D., E. R. Jacobson, L. W. Chang, C. Sanders, M. G. Hawkins, D. S. Guzman, T. Drazenovich, F. Dunker, E. K. Kamaka, D. Fisher, D. R. Reavill, L. F. Meola, G. Levens, and J. L. DeRisi.** 2015. Widespread recombination, reassortment, and transmission of unbalanced compound viral genotypes in natural arenavirus infections. PLoS Pathog. **11:**e1004900. doi: 10.1371/journal.ppat.1004900 [doi].

9. **Hepojoki, J., P. Salmenpera, T. Sironen, U. Hetzel, Y. Korzyukov, A. Kipar, and O. Vapalahti.** 2015. Arenavirus Coinfections Are Common in Snakes with Boid Inclusion Body Disease. J. Virol. **89:**8657-8660. doi: 10.1128/JVI.01112-15 [doi].

10. **Stenglein, M. D., D. Sanchez-Migallon Guzman, V. E. Garcia, M. L. Layton, L. L. Hoon-Hanks, S. M. Boback, M. K. Keel, T. Drazenovich, M. G. Hawkins, and J. L. DeRisi.** 2017. Differential Disease Susceptibilities in Experimentally Reptarenavirus-Infected Boa Constrictors and Ball Pythons. J. Virol. **91:**10.1128/JVI.00451-17. Print 2017 Aug 1. doi: e00451-17 [pii].

11. **Chang, L. W., A. Fu, E. Wozniak, M. Chow, D. G. Duke, L. Green, K. Kelley, J. A. Hernandez, and E. R. Jacobson.** 2013. Immunohistochemical detection of a unique protein within cells of snakes having inclusion body disease, a world-wide disease seen in members of the families Boidae and Pythonidae. PLoS One. **8:**e82916. doi: 10.1371/journal.pone.0082916 [doi].

12. **Chang, L., D. Fu, M. D. Stenglein, J. A. Hernandez, J. L. DeRisi, and E. R. Jacobson.** 2016. Detection and prevalence of boid inclusion body disease in collections of boas and pythons using immunological assays. Vet. J. **218:**13-18. doi: S1090-0233(16)30172-1 [pii].

13. **Windbichler, K., E. Michalopoulou, P. Palamides, T. Pesch, C. Jelinek, O. Vapalahti, A. Kipar, U. Hetzel, and J. Hepojoki.** 2019. Antibody response in snakes with boid inclusion body disease. PLoS One. **14:**e0221863. doi: 10.1371/journal.pone.0221863 [doi].

14. **Keilwerth, M., I. Buhler, R. Hoffmann, H. Soliman, and M. El-Matbouli.** 2012. Inclusion Body Disease (IBD of Boids)--a haematological, histological and electron microscopical study. Berl. Munch. Tierarztl. Wochenschr. **125:**411-417.

15. **Aqrawi, T., A. C. Stohr, T. Knauf-Witzens, A. Krengel, K. O. Heckers, and R. E. Marschang.** 2015. Identification of snake arenaviruses in live boas and pythons in a zoo in Germany. Tierarztl. Prax. Ausg. K. Kleintiere Heimtiere. **43:**239-247. doi: 140743 [pii].

16. **Radoshitzky, S. R., Y. Bao, M. J. Buchmeier, R. N. Charrel, A. N. Clawson, C. S. Clegg, J. L. DeRisi, S. Emonet, J. P. Gonzalez, J. H. Kuhn, I. S. Lukashevich, C. J. Peters, V. Romanowski, M. S. Salvato, M. D. Stenglein, and J. C. de la Torre.** 2015. Past, present, and future of arenavirus taxonomy. Arch. Virol. **160:**1851-1874. doi: 10.1007/s00705-015-2418-y [doi].

17. **Radoshitzky, S. R., M. J. Buchmeier, R. N. Charrel, J. C. S. Clegg, J. J. Gonzalez, S. Gunther, J. Hepojoki, J. H. Kuhn, I. S. Lukashevich, V. Romanowski, M. S. Salvato, M. Sironi, M. D. Stenglein, J. C. de la Torre, and C. ICTV Report.** 2019. ICTV Virus Taxonomy Profile: Arenaviridae. J. Gen. Virol. **100:**1200-1201. doi: 10.1099/jgv.0.001280 [doi].

18. **Hepojoki, J., S. Hepojoki, T. Smura, L. Szirovicza, E. Dervas, B. Prahauser, L. Nufer, E. M. Schraner, O. Vapalahti, A. Kipar, and U. Hetzel.** 2018. Characterization of Haartman Institute snake virus-1 (HISV-1) and HISV-like viruses-The representatives of genus Hartmanivirus, family Arenaviridae. PLoS Pathog. **14:**e1007415. doi: 10.1371/journal.ppat.1007415 [doi].

19. **Maes, P., S. V. Alkhovsky, Y. Bao, M. Beer, M. Birkhead, T. Briese, M. J. Buchmeier, C. H. Calisher, R. N. Charrel, I. R. Choi, C. S. Clegg, J. C. de la Torre, E. Delwart, J. L. DeRisi, P. L. Di Bello, F. Di Serio, M. Digiaro, V. V. Dolja, C. Drosten, T. Z. Druciarek, J. Du, H. Ebihara, T. Elbeaino, R. C. Gergerich, A. N. Gillis, J. J. Gonzalez, A. L. Haenni, J. Hepojoki, U. Hetzel, T. Ho, N. Hong, R. K. Jain, P. Jansen**

van Vuren, Q. Jin, M. G. Jonson, S. Junglen, K. E. Keller, A. Kemp, A. Kipar, N. O. Kondov, E. V. Koonin, R. Kormelink, Y. Korzyukov, M. Krupovic, A. J. Lambert, A. G. Laney, M. LeBreton, I. S. Lukashevich, M. Marklewitz, W. Markotter, G. P. Martelli, R. R. Martin, N. Mielke-Ehret, H. P. Muhlbach, B. Navarro, T. F. F. Ng, M. R. T. Nunes, G. Palacios, J. T. Paweska, C. J. Peters, A. Plyusnin, S. R. Radoshitzky, V. Romanowski, P. Salmenpera, M. S. Salvato, H. Sanfacon, T. Sasaya, C. Schmaljohn, B. S. Schneider, Y. Shirako, S. Siddell, T. A. Sironen, M. D. Stenglein, N. Storm, H. Sudini, R. B. Tesh, I. E. Tzanetakis, M. Uppala, O. Vapalahti, N. Vasilakis, P. J. Walker, G. Wang, L. Wang, Y. Wang, T. Wei, M. R. Wiley, Y. I. Wolf, N. D. Wolfe, Z. Wu, W. Xu, L. Yang, Z. Yang, S. D. Yeh, Y. Z. Zhang, Y. Zheng, X. Zhou, C. Zhu, F. Zirkel, and J. H. Kuhn. 2018. Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. Arch. Virol. . doi: 10.1007/s00705-018-3843-5 [doi].

20. **Abudurexiti, A., S. Adkins, D. Alioto, S. V. Alkhovsky, T. Avsic-Zupanc, M. J. Ballinger, D. A. Bente, M. Beer, E. Bergeron, C. D. Blair, T. Briese, M. J. Buchmeier, F. J. Burt, C. H. Calisher, C. Chang, R. N. Charrel, I. R. Choi, J. C. S. Clegg, J. C. de la Torre, X. de Lamballerie, F. Deng, F. Di Serio, M. Digiaro, M. A. Drebot, X. Duan, H. Ebihara, T. Elbeaino, K. Ergunay, C. F. Fulhorst, A. R. Garrison, G. F. Gao, J. J. Gonzalez, M. H. Groschup, S. Gunther, A. L. Haenni, R. A. Hall, J. Hepojoki, R. Hewson, Z. Hu, H. R. Hughes, M. G. Jonson, S. Junglen, B. Klempa, J. Klingstrom, C. Kou, L. Laenen, A. J. Lambert, S. A. Langevin, D. Liu, I. S. Lukashevich, T. Luo, C. Lu, P. Maes, W. M. de Souza, M. Marklewitz, G. P. Martelli, K. Matsuno, N. Mielke-Ehret, M. Minutolo, A. Mirazimi, A. Moming, H. P. Muhlbach, R. Naidu, B. Navarro, M. R. T. Nunes, G. Palacios, A. Papa, A. Pauvolid-Correa, J. T. Paweska, J. Qiao, S. R. Radoshitzky, R. O. Resende, V. Romanowski, A. A. Sall, M. S. Salvato, T. Sasaya, S. Shen, X. Shi, Y. Shirako, P. Simmonds, M. Sironi, J. W. Song, J. R. Spengler, M. D. Stenglein, Z. Su, S. Sun, S. Tang, M. Turina, B. Wang, C. Wang, H. Wang, J. Wang, T.**

Wei, A. E. Whitfield, F. M. Zerbini, J. Zhang, L. Zhang, Y. Zhang, Y. Z. Zhang, Y. Zhang, X. Zhou, L. Zhu, and J. H. Kuhn. 2019. Taxonomy of the order Bunyavirales: update 2019. Arch. Virol. **164:**1949-1965. doi: 10.1007/s00705-019-04253-6 [doi].

21. **Turchetti, A., H. Tinoco, M. de Campos Cordeiro Malta, E. Loyola Teixeira da Costa, A. Pessanha, S. Soave, A. Paixao, and R. Santos.** 2013. Inclusion body disease in a *Corallus hortulanus*. Braz J Vet Pathol. **6:**15-18.

22. **Keller, S., U. Hetzel, T. Sironen, Y. Korzyukov, O. Vapalahti, A. Kipar, and J. Hepojoki.** 2017. Co-infecting Reptarenaviruses Can Be Vertically Transmitted in Boa Constrictor. PLoS Pathog. **13:**e1006179. doi: 10.1371/journal.ppat.1006179 [doi].

23. **Dervas, E., J. Hepojoki, A. Laimbacher, F. Romero-Palomo, C. Jelinek, S. Keller, T. Smura, S. Hepojoki, A. Kipar, and U. Hetzel.** 2017. Nidovirus-Associated Proliferative Pneumonia in the Green Tree Python (Morelia viridis). J. Virol. . doi: JVI.00718-17 [pii].

24. **Hetzel, U., L. Szirovicza, T. Smura, B. Prahauser, O. Vapalahti, A. Kipar, and J. Hepojoki.** 2019. Identification of a Novel Deltavirus in Boa Constrictors. MBio. **10:**10.1128/mBio.00014-19. doi: e00014-19 [pii].

25. **Shi, M., X. D. Lin, X. Chen, J. H. Tian, L. J. Chen, K. Li, W. Wang, J. S. Eden, J. J. Shen, L. Liu, E. C. Holmes, and Y. Z. Zhang.** 2018. The evolutionary history of vertebrate RNA viruses. Nature. **556:**197-202. doi: 10.1038/s41586-018-0012-7 [doi].

26. **Pees, M., V. Schmidt, R. E. Marschang, O. Heckers, J. Thielebein, J. Seybold, and M. E. Krautwald.** 2009. Untersuchungen auf infektiöse Erkrankungen in unauffälligen Boiden-Beständen. 1. DVG-Tagung Über Vogel- Und Reptilienkrankeiten. 33-38.

27. **Grego, K. F., M. P. N. d. Carvalho, M. P. V. Cunha, T. Knöbl, F. C. Pogliani, J. L. Catão-Dias, S. S. Sant'Anna, M. S. Ribeiro, and F. P. Sellera.** 2017. Antimicrobial photodynamic therapy for infectious stomatitis in snakes: Clinical views and microbiological findings. Photodiagnosis and Photodynamic Therapy. **20:**196-200. doi: https://doi.org/10.1016/j.pdpdt.2017.10.004 ".

549 28. **dos Santos, G. S., E. G. Solidônio, M. C. V. V. Costa, R. O. A. Melo, I. F. A. C. de**
550 **Souza, G. R. Silva, and K. X. F. R. Sena.** 2015. Study of the Enterobacteriaceae Group
551 CESP (Citrobacter, Enterobacter, Serratia, Providencia, Morganella and Hafnia): A Review,
552 p. 794-805. *In* A. Méndez-Vilas (ed.), The Battle Against Microbial Pathogens: Basic
553 Science, Technological Advances and Educational Programs. Formatex Research Center S.L.,
554 Spain.
555 29. **GIBBON, J. W., D. E. SCOTT, T. J. RYAN, K. A. BUHLMANN, T. D.**
556 **TUBERVILLE, B. S. METTS, J. L. GREENE, T. MILLS, Y. LEIDEN, S. POPPY, and**
557 **C. T. WINNE.** 2000. Reptile species are declining on a global scale. Six significant threats to
558 reptile populations are habitat loss and degradation, introduced invasive species,
559 environmental pollution, disease, unsustainable use, and global climate change; The Global
560 Decline of Reptiles, Déjà Vu Amphibians. Bioscience. **50:**653-666. doi: 10.1641/0006-
561 3568(2000)050[0653:tgdord]2.0.co;2.
562 30. **Hepojoki, J., A. Kipar, Y. Korzyukov, L. Bell-Sakyi, O. Vapalahti, and U. Hetzel.**
563 2015. Replication of Boid Inclusion Body Disease-Associated Arenaviruses Is Temperature
564 Sensitive in both Boid and Mammalian Cells. J. Virol. **89:**1119-1128. doi:
565 10.1128/JVI.03119-14 [doi].
566 31. **Katoh, K., and D. M. Standley.** 2013. MAFFT Multiple Sequence Alignment Software
567 Version 7: Improvements in Performance and Usability. Mbe. **30:**772-780. doi:
568 10.1093/molbev/mst010.
569 32. **Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Hohna, B.**
570 **Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck.** 2012. MrBayes 3.2: efficient
571 Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol.
572 **61:**539-542. doi: 10.1093/sysbio/sys029 [doi].
573

Table 1: Animals and pathological findings. All animals were *Boa constrictor constrictor* snakes held in captivity in Porto Alegre, Brazil.

Case No	Age	Sex	Origin	Captivity	Clinical history	Diagnoses
[1]	12 y	F	Novo Arão (Amazonas, Brazil)	Private owner	Regurgitation and ulcerative lesion in oral cavity for 3 mo. Antibiotic therapy, fluid therapy, catheter feeding for 2 mo; no improvement, then apathy and death.	Chronic ulcerative stomatitis and osteomyelitis (maxilla); chronic ulcerative dermatitis and myositis; BIBD
[2]	10 y	F	Novo Arão (Amazonas, Brazil)	Private owner	Found dead without prior clinical signs.	Fibrinonecrotizing cloacitis, embolic-metastatic nephritis; hepatic lipidosis; BIBD
[3]	12 y	M	Novo Arão (Amazonas, Brazil)	Private owner	Apathy and neurological signs (eg. disorientation, star-gazing) for 5 mo; euthanasia	Focally ulcerated granulomatous stomatitis and osteomyelitis (maxilla); BIBD
[4]	13 y	M	Unknown	Botanic Zoo Foundation of Rio Grande	Oral mucosal bleeding, nasal discharge, lethargy, anorexia for appr. 5 mo; hospitalization and antibiotic therapy, fluid therapy, catheter feeding; no improvement; after 45 d apathy and death.	Emaciation; chronic suppurative sinusitis; BIBD

Snake No.	Virus name (abbreviation)	Segment	Contig length	GenBank accession no.	Average coverage	Closest match by PASC (% identity. GenBank accession no.)
1	Aramboia boa virus-1 (ArBV-1)	S	3372	MN567042	14173.9	74.0%. KP071473.1 Unclassified Reptarenavirus
1	Aramboia boa virus-1 (ArBV-1)	L	6883	MN567044	5232.6	72.7%. KR870030.1 University of Helsinki reptarenavirus
1	Kaltenbach virus-1 (KaBV-1)	L	7010	MN567043	7105.8	72.4%. KP071677.1 Unclassified Reptarenavirus
1	Arabuta Snake virus-1 (ArSV-1)	L	6780	MN567045	1413.6	63.3%. KR870020.1 University of Helsinki reptarenavirus
1	University of Helsinki virus-2 (UHV-2)	L	6813	MN567046	2765.8	86.4%. KR870030.1 University of Helsinki reptarenavirus
1	Frankfurter Strasse virus-1 (FStV-1)	L	6831	MN567047	2233.2	74.1%. KR870030.1 University of Helsinki reptarenavirus
1	SetPatVet virus-1 (SPVV-1)	S	3560	MN567048	268.3	21.8%. FJ607031.1 Lymphocytic choriomeningitis mammarenavirus
1	SetPatVet virus-1 (SPVV-1)	L	3578 and 2223	MN567049 and MN567050	35.1	N.A.
1	Herr Frank virus-1 (HFrV-1)	N.A.	10718	MN567051	1698.9	N.A.
2	Aramboia boa virus-1 (ArBV-1)	S	3372	MN567052	26110.1	73.7%. KP071473.1 Unclassified Reptarenavirus
2	Aramboia boa virus-1 (ArBV-1)	L	6948	MN567054	9586.7	72.8%. KR870030.1 University of Helsinki reptarenavirus
2	Kaltenbach virus-1 (KaBV-1)	L	7009	MN567053	6688.2	72.3%. KP071567.1 Unclassified Reptarenavirus
2	Andere Heimat virus-1 (AHeV-1)	S	3534	MN567055	99.3	22.0%. FJ607031.1 Lymphocytic choriomeningitis mammarenavirus
2	Andere Heimat virus-1 (AHeV-1)	L	5953	MN567056	28.5	18.7%. MG812678.1 Lassa mammarenavirus
2	Herr Frank virus-1 (HFrV-1)	N.A.	10549	MN567057	1073.9	N.A.
3	Aramboia boa virus-1 (ArBV-1)	S	3351	MN567058	11236.5	74.0%. KP071671.1 Unclassified Reptarenavirus

3	Aramboia boa virus-1 (ArBV-1)	L	6926	MN567060	3443.9	72.7%. KR870030.1 University of Helsinki reptarenavirus
3	Kaltenbach virus-1 (KaBV-1)	L	7007	MN567059	2913.8	72.2%. KR870030.1 University of Helsinki reptarenavirus
3	Andere Heimat virus-1 (AHeV-1)	S	3598	MN567061	1433.3	21.8%. NC_004294.1 Lymphocytic choriomeningitis mammarenavirus
3	Andere Heimat virus-1 (AHeV-1)	L	5961	MN567062	548.3	18.9%. MG812678.1 Lassa mammarenavirus
3	Herr Frank virus-1 (HFrV-1)	N.A.	10574	MN567063	126.8	N.A.
4	Porto Alegre virus-1 (PAV-1)	S	3464	MN567064	2071.6	73.3%. KP071559.1 Unclassified Reptarenavirus
4	Porto Alegre virus-1 (PAV-1)	L	6911	MN567065	1158.3	79.6%. KP071549.1 Unclassified Reptarenavirus
4	Saudades virus-1 (SauV-1)	L	6835	MN567066	523.1	73.2%. KP071479.1 Unclassified Reptarenavirus
4	Gaucho virus-1 (GauV-1)	L	7203	MN567067	438.3	72.8%. KR870030.1 University of Helsinki reptarenavirus

A) The nucleotide identities between reptarenavirus L segments identified in this study.

Animal [no.], virus segment	[1], ArBV-1 L seg.	[2], ArBV-1 L seg.	[3], ArBV-1 L seg.	[1], KaBV-1 L seg.	[2], KaBV-1 L seg.	[3], KaBV-1 L seg.	[1], ArSV-1 L seg.	[1], UHV-2 L seg.	[1], FStV-1 L seg.	[4], PAV L seg.	[4], SauV-1 L seg.	[4], GauV-1 L seg.
[1], ArBV-1 L seg.												
[2], ArBV-1 L seg.	99 %											
[3], ArBV-1 L seg.	99 %	100 %										
[1], KaBV-1 L seg.	71 %	71 %	71 %									
[2], KaBV-1 L seg.	71 %	71 %	71 %	100 %								
[3], KaBV-1 L seg.	71 %	72 %	71 %	100 %	100 %							
[1], ArSV-1 L seg.	63 %	64 %	64 %	63 %	63 %	63 %						
[1], UHV-2 L seg.	73 %	74 %	74 %	73 %	73 %	73 %	63 %					
[1], FStV-1 L seg.	73 %	73 %	73 %	73 %	73 %	73 %	63 %	74 %				
[4], PAV L seg.	72 %	73 %	73 %	72 %	72 %	72 %	64 %	77 %	74 %			
[4], SauV-1 L seg.	73 %	73 %	73 %	72 %	72 %	72 %	63 %	73 %	72 %	73 %		
[4], GauV-1 L seg.	69 %	69 %	69 %	70 %	70 %	70 %	60 %	70 %	70 %	71 %	68 %	

B) The nucleotide identities between reptarenavirus S segments identified in this study.

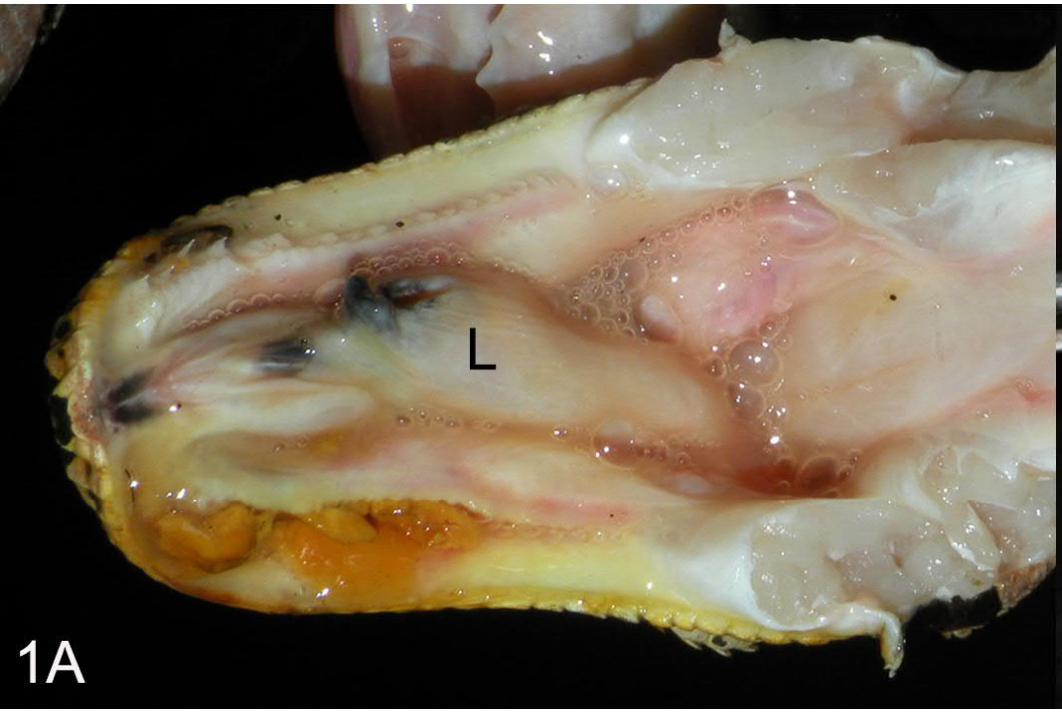
Animal [no.], virus segment	[1], ArBV-1 S seg.	[2], ArBV-1 S seg.	[3], ArBV-1 S seg.	[4], PAV S seg.
[1], ArBV-1 S seg.				
[2], ArBV-1 S seg.	97 %			
[3], ArBV-1 S seg.	96 %	99 %		
[4], PAV S seg.	72 %	73 %	73 %	

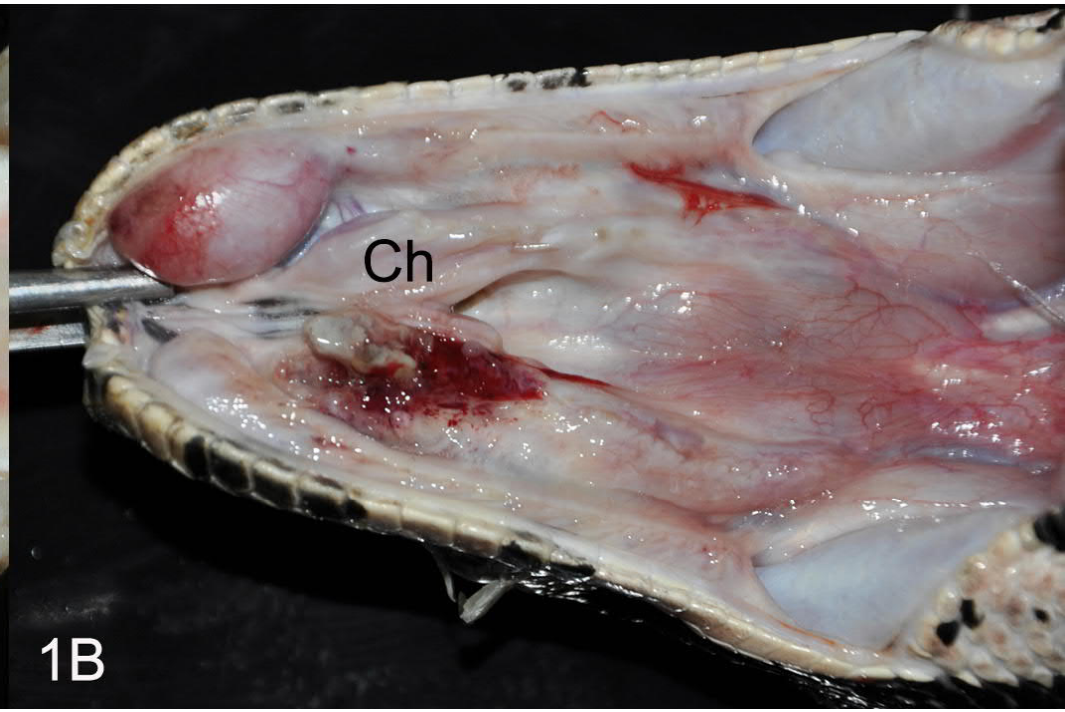
A) The nucleotide identities between hartmanivirus L segments identified in this and earlier studies.

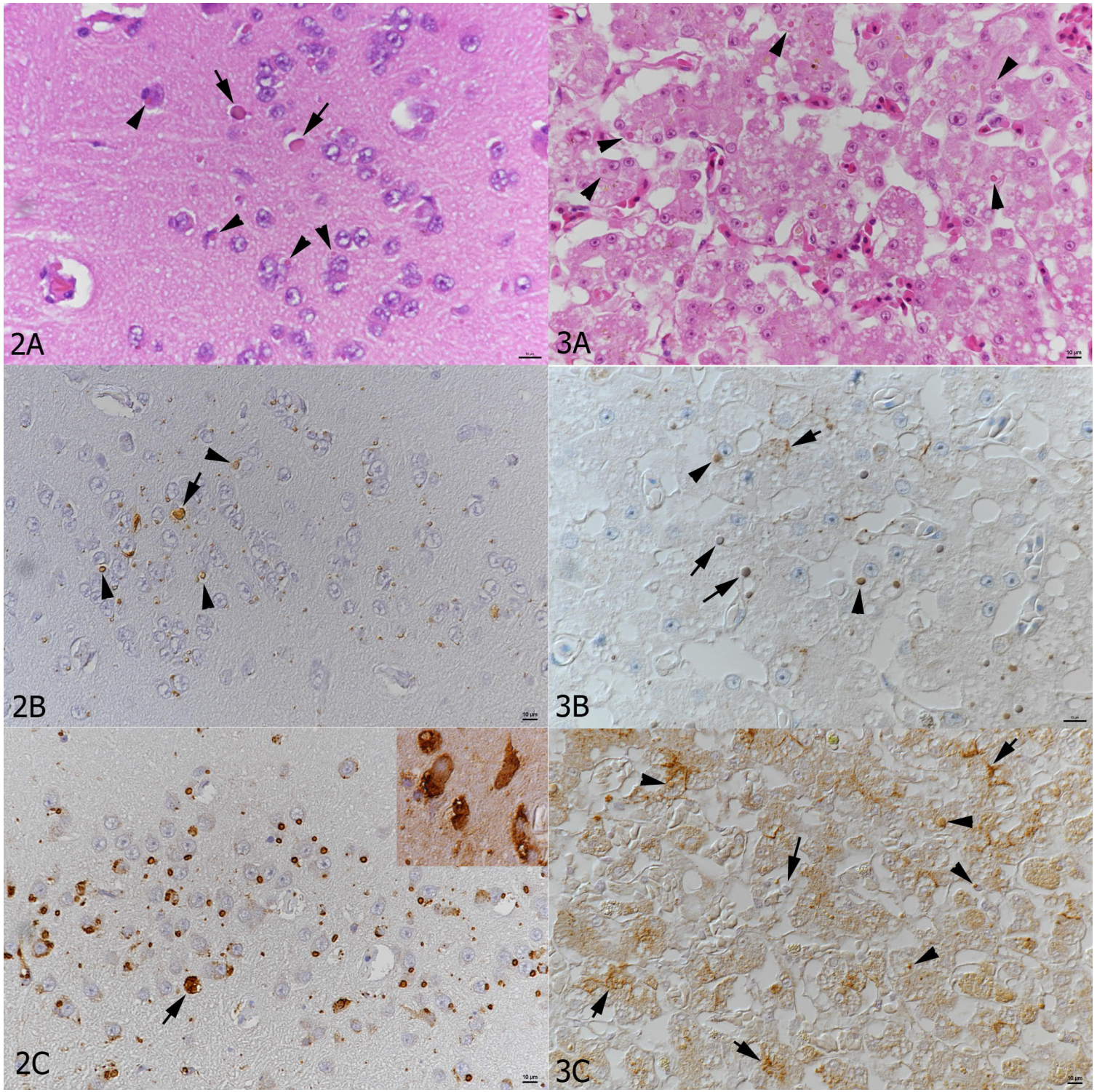
Animal [no.], virus segment	[1], SPVV-1 L seg. piece1/2	[1], SPVV-1 L seg. piece2/2	[2], AHeV-1 L seg.	[3], AHeV-1 L seg.	HISV-1, NC_043443.1	HISV-2, MH483042.1	OScV-1, MH483028.1	OScV-2, MH483025.1	VPZV-1, MH483040.1	VPZV-2, MH483044.1	DaMV-1, MH778629.1
[1], SPVV-1 L seg. piece1/2											
[1], SPVV-1 L seg. piece2/2	2 %										
[2], AHeV-1 L seg.	83 %	70 %									
[3], AHeV-1 L seg.	83 %	82 %	100 %								
HISV-1, NC_043443.1	60 %	56 %	59 %	59 %							
HISV-2, MH483042.1	61 %	56 %	59 %	60 %	89 %						
OScV-1, MH483028.1	62 %	55 %	59 %	59 %	65 %	65 %					
OScV-2, MH483025.1	61 %	56 %	59 %	59 %	65 %	65 %	85 %				
VPZV-1, MH483040.1	61 %	58 %	60 %	60 %	65 %	65 %	65 %	65 %			
VPZV-2, MH483044.1	61 %	56 %	59 %	59 %	65 %	65 %	64 %	64 %	86 %		
DaMV-1, MH778629.1	60 %	56 %	58 %	59 %	69 %	69 %	65 %	65 %	65 %	65 %	

B) The nucleotide identities between hartmanivirus S segments identified in this and earlier studies.

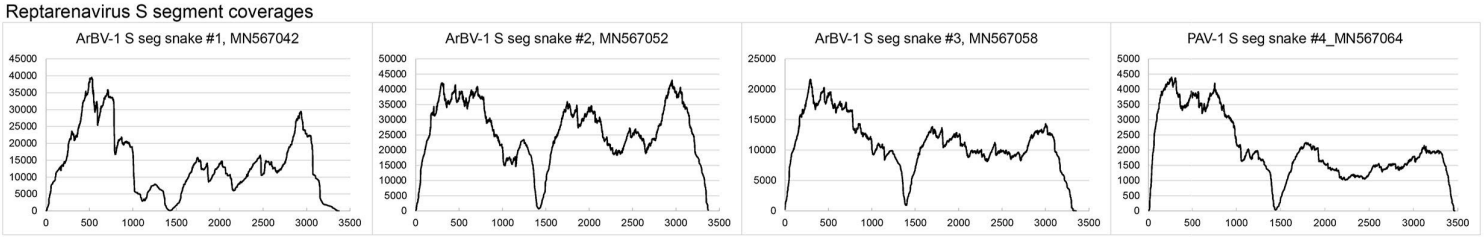
Animal [no.], virus segment	[1], SPVV-1 S seg.	[2], AHeV-1 S seg.	[3], AHeV-1 S seg.	HISV-1, NC_043444.1	HISV-2, MH483041.1	OScV-1, MH483027.1	OScV-2, MH483029.1	VPZV-1, MH483039.1	VPZV-2, MH483043.1	DaMV-1, MH483026.1
[1], SPVV-1 S seg.										
[2], AHeV-1 S seg.	77 %									
[3], AHeV-1 S seg.	77 %	100 %								
HISV-1, NC_043444.1	52 %	53 %	54 %							
HISV-2, MH483041.1	53 %	53 %	54 %	86 %						
OScV-1, MH483027.1	52 %	53 %	53 %	61 %	61 %					
OScV-2, MH483029.1	53 %	52 %	52 %	61 %	61 %	79 %				
VPZV-1, MH483039.1	51 %	51 %	52 %	62 %	62 %	60 %	60 %			
VPZV-2, MH483043.1	51 %	52 %	52 %	62 %	62 %	60 %	59 %	84 %		
DaMV-1, MH483026.1	52 %	52 %	53 %	65 %	65 %	60 %	63 %	61 %	61 %	


L
1A

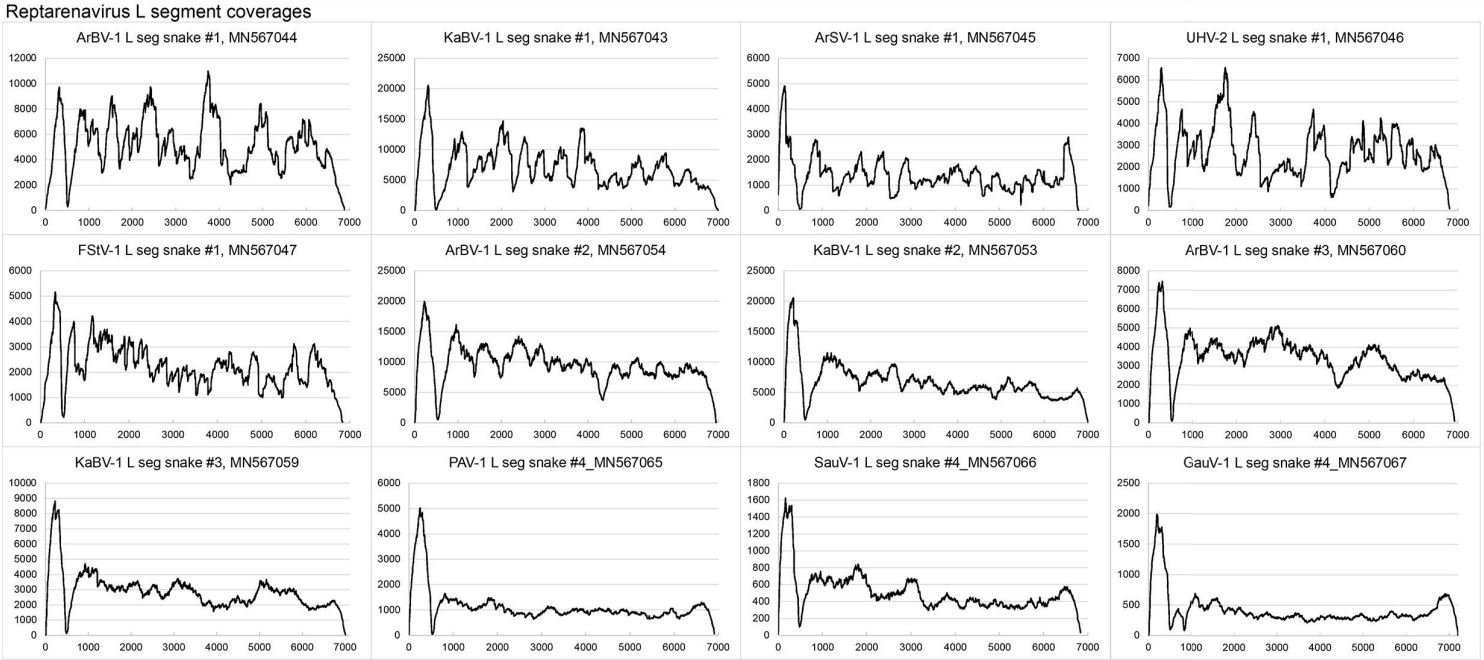

Ch
1B


2A
3A
2B
3B
2C
3C

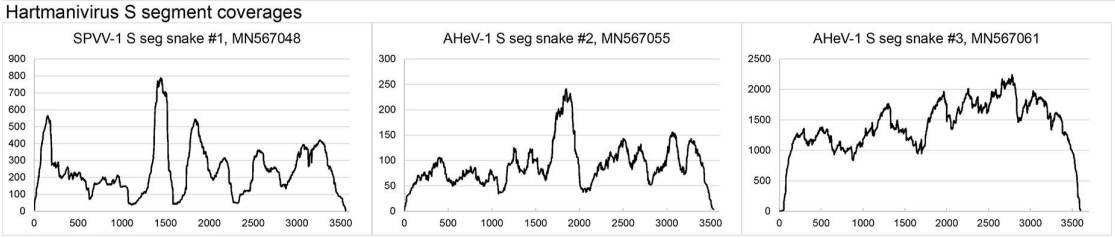
Reptarenavirus S segment coverages



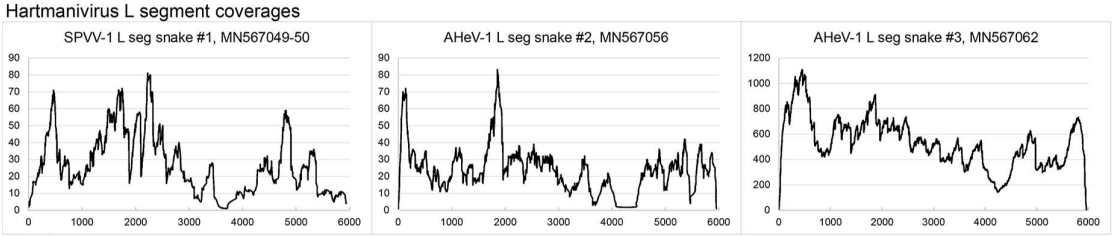
Reptarenavirus L segment coverages



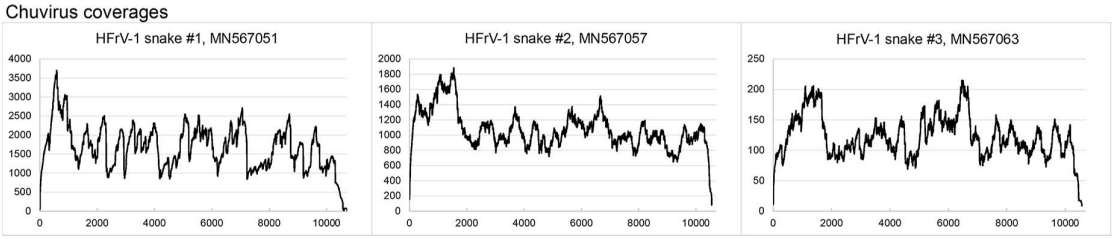
Hartmanivirus S segment coverages

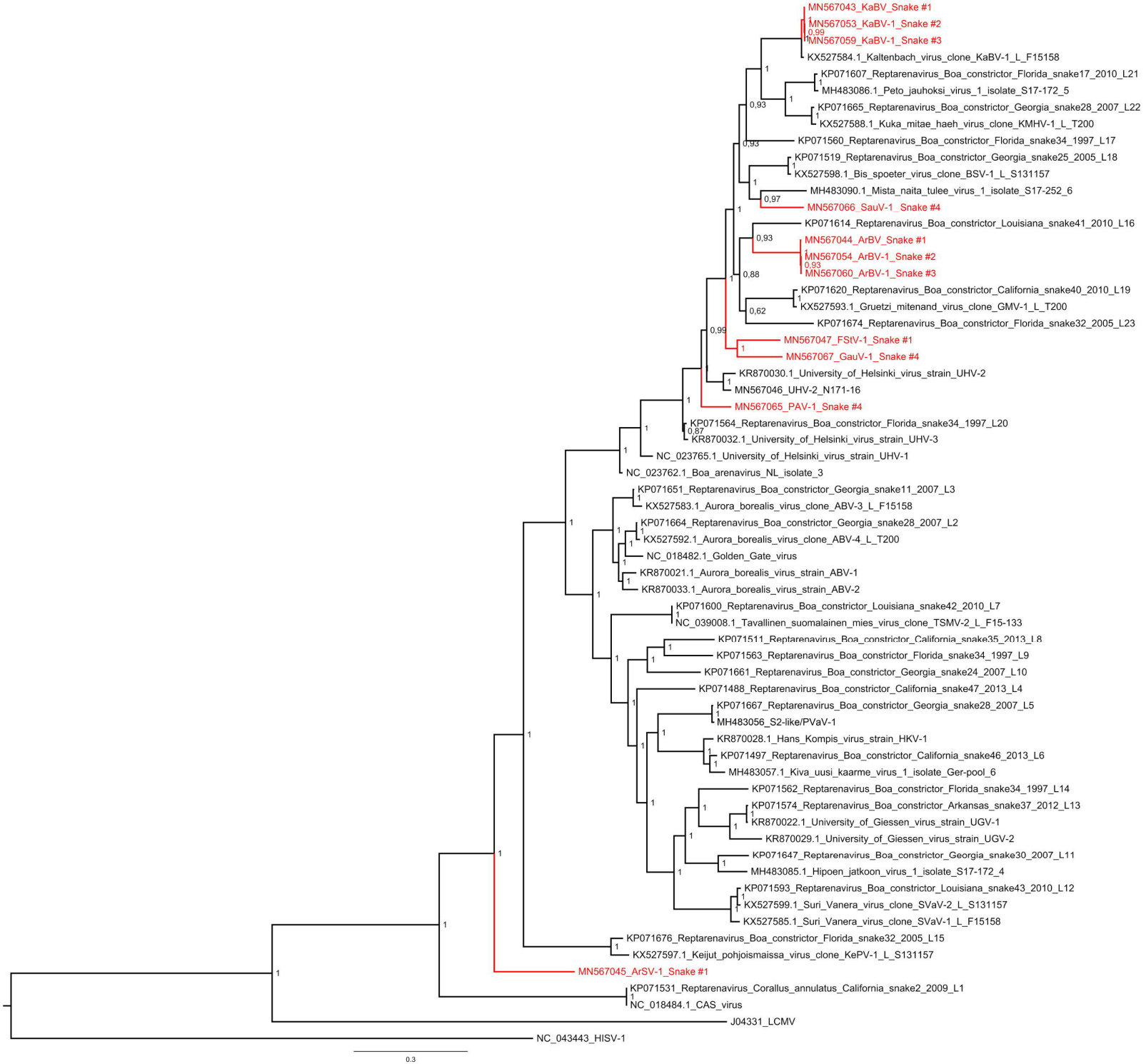


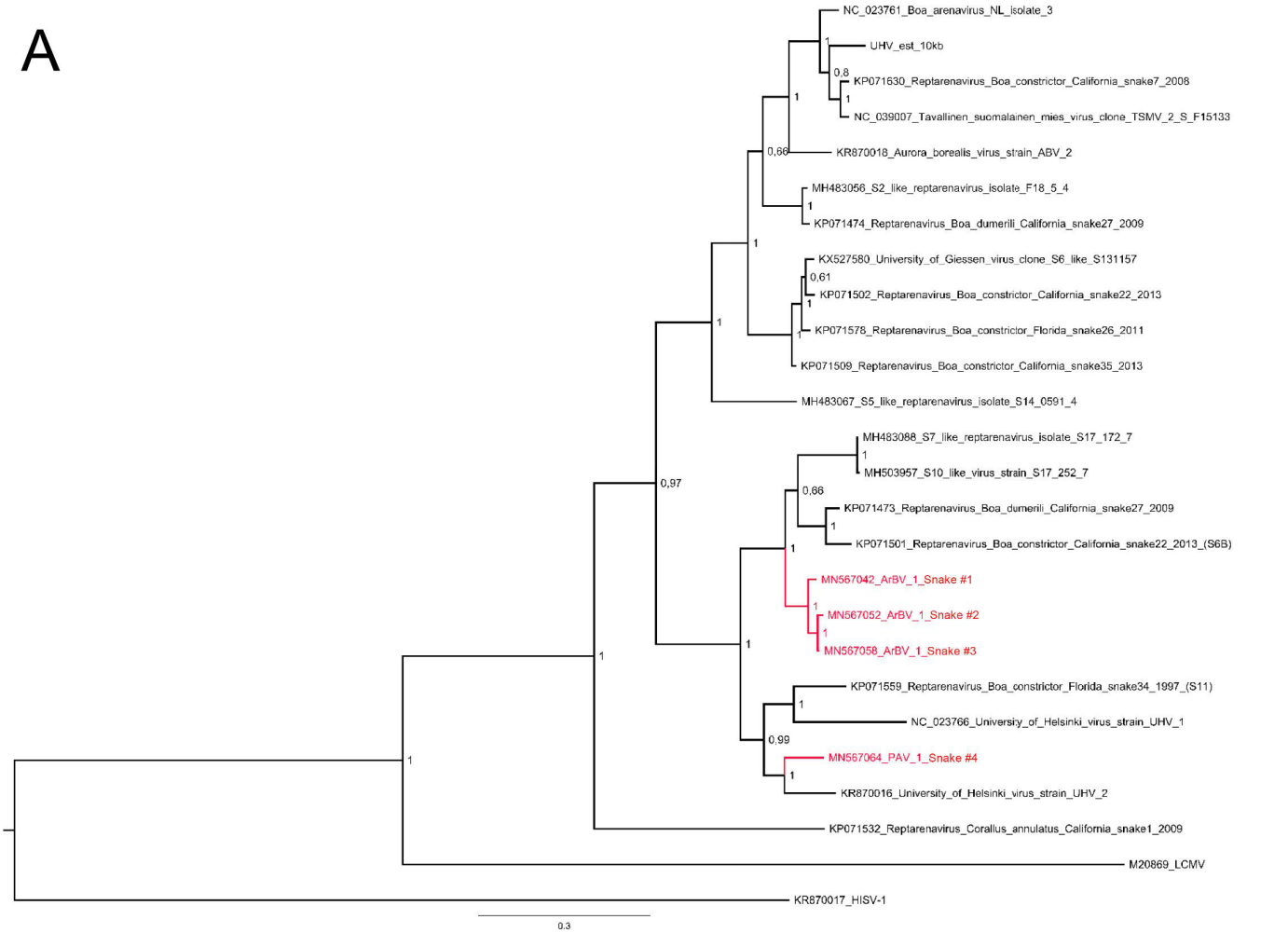
Hartmanivirus L segment coverages

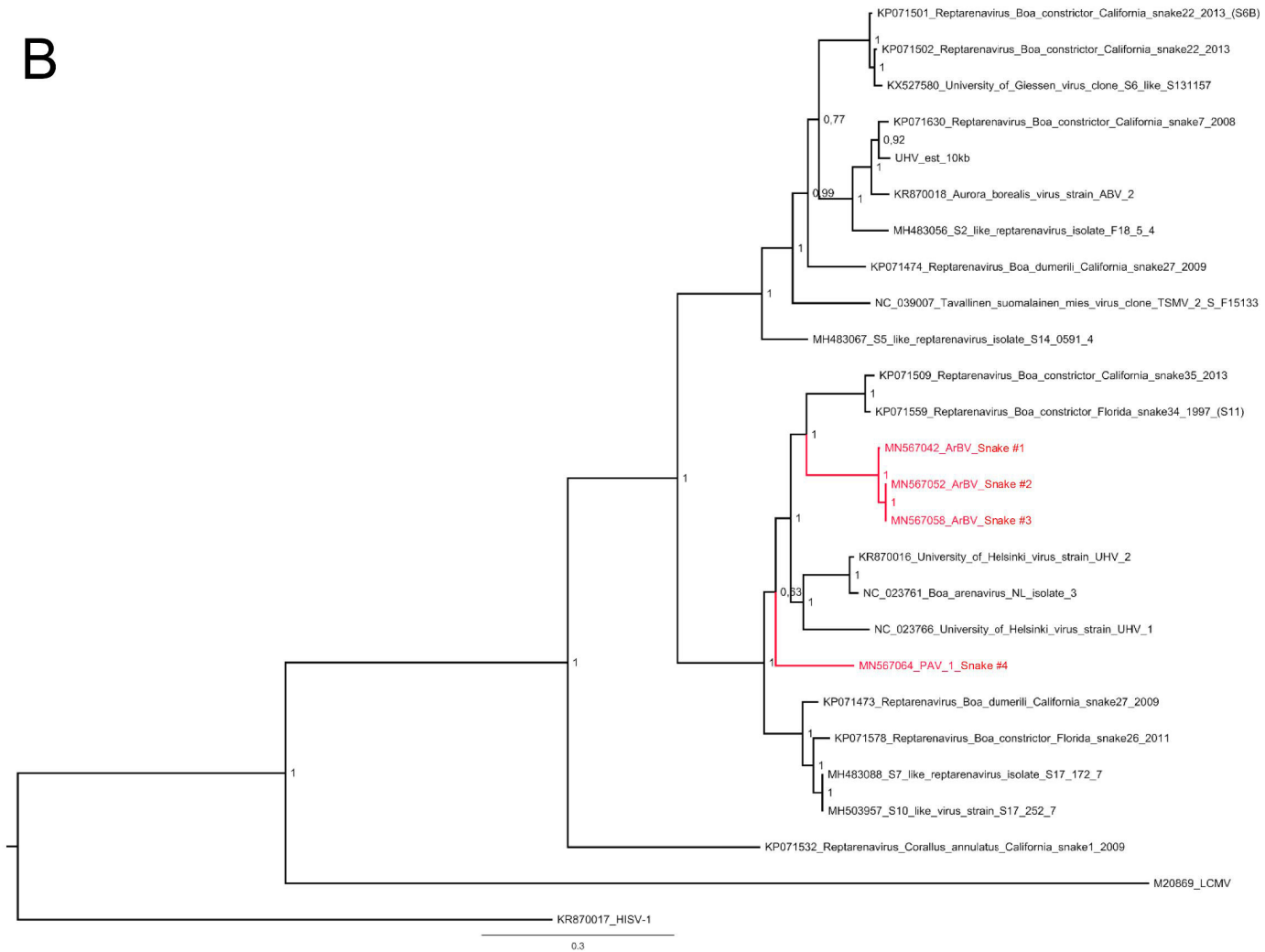


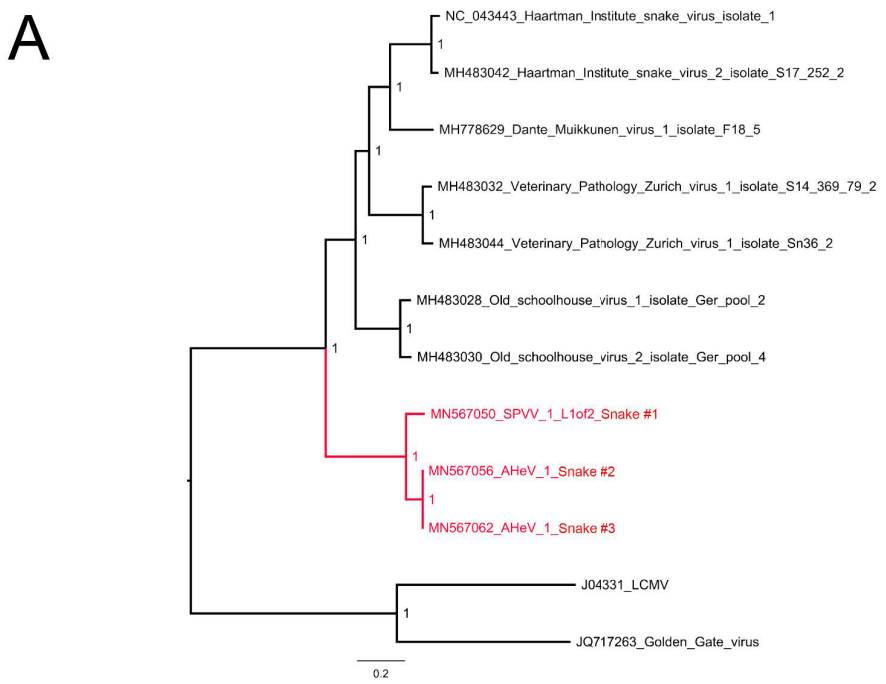
Chuvirus coverages

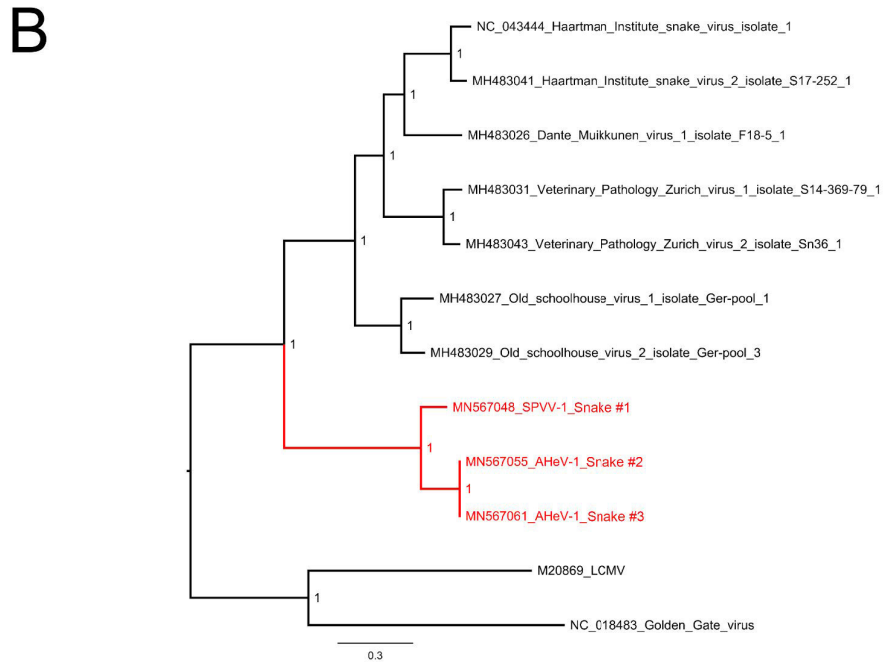


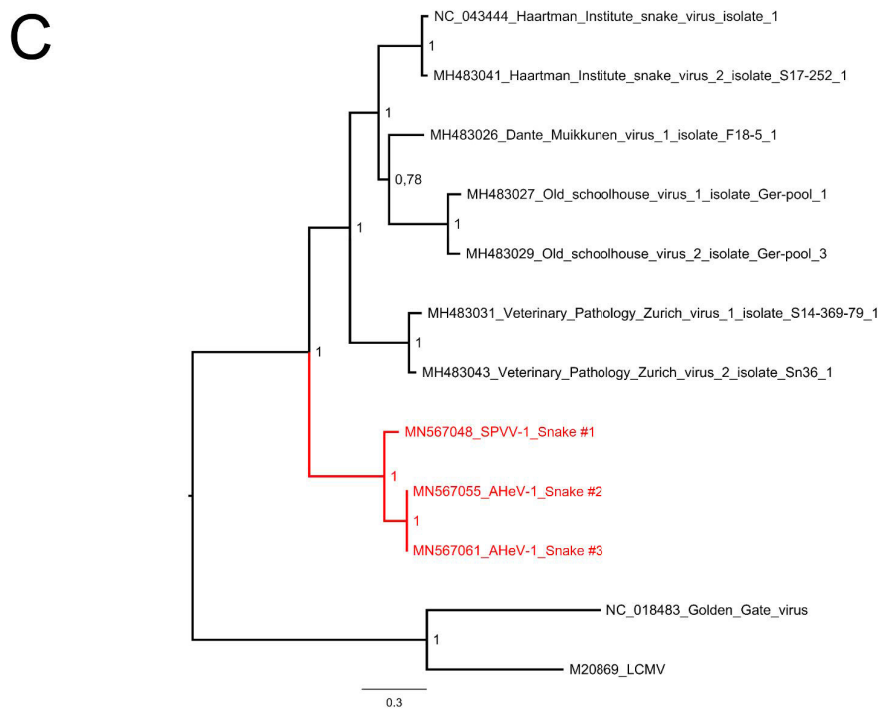

MN567043_KaBV_Snake #1
MN567053_KaBV-1_Snake #2
MN567059_KaBV-1_Snake #3
KX527584.1_Kaltenbach_virus_clone_KaBV-1_L_F15158
KP071607_Reptarenavirus_Boa_constrictor_Florida_snake17_2010_L21
MH483086.1_Peto_jauhoksi_virus_1_isolate_S17-172_5
KP071665_Reptarenavirus_Boa_constrictor_Georgia_snake28_2007_L22
KX527588.1_Kuka_mitae_haeh_virus_clone_KMHV-1_L_T200
KP071560_Reptarenavirus_Boa_constrictor_Florida_snake34_1997_L17
KP071519_Reptarenavirus_Boa_constrictor_Georgia_snake25_2005_L18
KX527598.1_Bis_spoeter_virus_clone_BSV-1_L_S131157
MH483090.1_Mista_naita_tulee_virus_1_isolate_S17-252_6
MN567066_SauV-1_Snake #4
KP071614_Reptarenavirus_Boa_constrictor_Louisiana_snake41_2010_L16
MN567044_ArBV_Snake #1
MN567054_ArBV-1_Snake #2
MN567060_ArBV-1_Snake #3
KP071620_Reptarenavirus_Boa_constrictor_California_snake40_2010_L19
KX527593.1_Gruetzi_mitenand_virus_clone_GMV-1_L_T200
KP071674_Reptarenavirus_Boa_constrictor_Florida_snake32_2005_L23
MN567047_FStV-1_Snake #1
MN567067_GauV-1_Snake #4
KR870030.1_University_of_Helsinki_virus_strain_UHV-2
MN567046_UHV-2_N171-16
MN567065_PAV-1_Snake #4
KP071564_Reptarenavirus_Boa_constrictor_Florida_snake34_1997_L20
KR870032.1_University_of_Helsinki_virus_strain_UHV-3
NC_023765.1_University_of_Helsinki_virus_strain_UHV-1
NC_023762.1_Boa_arenavirus_NL_isolate_3
KP071651_Reptarenavirus_Boa_constrictor_Georgia_snake11_2007_L3
KX527583.1_Aurora_borealis_virus_clone_ABV-3_L_F15158
KP071664_Reptarenavirus_Boa_constrictor_Georgia_snake28_2007_L2
KX527592.1_Aurora_borealis_virus_clone_ABV-4_L_T200
NC_018482.1_Golden_Gate_virus
KR870021.1_Aurora_borealis_virus_strain_ABV-1
KR870033.1_Aurora_borealis_virus_strain_ABV-2
KP071600_Reptarenavirus_Boa_constrictor_Louisiana_snake42_2010_L7
NC_039008.1_Tavallinen_suomalainen_mies_virus_clone_TSMV-2_L_F15-133
KP071511_Reptarenavirus_Boa_constrictor_California_snake35_2013_L8
KP071563_Reptarenavirus_Boa_constrictor_Florida_snake34_1997_L9
KP071661_Reptarenavirus_Boa_constrictor_Georgia_snake24_2007_L10
KP071488_Reptarenavirus_Boa_constrictor_California_snake47_2013_L4
KP071667_Reptarenavirus_Boa_constrictor_Georgia_snake28_2007_L5
MH483056_S2-like/PVaV-1
KR870028.1_Hans_Kompis_virus_strain_HKV-1
KP071497_Reptarenavirus_Boa_constrictor_California_snake46_2013_L6
MH483057.1_Kiva_uusi_kaarme_virus_1_isolate_Ger-pool_6
KP071562_Reptarenavirus_Boa_constrictor_Florida_snake34_1997_L14
KP071574_Reptarenavirus_Boa_constrictor_Arkansas_snake37_2012_L13
KR870022.1_University_of_Giessen_virus_strain_UGV-1
KR870029.1_University_of_Giessen_virus_strain_UGV-2
KP071647_Reptarenavirus_Boa_constrictor_Georgia_snake30_2007_L11
MH483085.1_Hipoen_jatkoon_virus_1_isolate_S17-172_4
KP071593_Reptarenavirus_Boa_constrictor_Louisiana_snake43_2010_L12
KX527599.1_Suri_Vanera_virus_clone_SVaV-2_L_S131157
KX527585.1_Suri_Vanera_virus_clone_SVaV-1_L_F15158
KP071676_Reptarenavirus_Boa_constrictor_Florida_snake32_2005_L15
KX527597.1_Keijut_pohjoismaissa_virus_clone_KePV-1_L_S131157
MN567045_ArSV-1_Snake #1
KP071531_Reptarenavirus_Corallus_annulatus_California_snake2_2009_L1
NC_018484.1_CAS_virus
J04331_LCMV
NC_043443_HISV-1
0.3


A
NC_023761_Boa_arenavirus_NL_isolate_3
UHV_est_10kb
KP071630_Reptarenavirus_Boa_constrictor_California_snake7_2008
NC_039007_Tavallinen_suomalainen_mies_virus_clone_TSMV_2_S_F15133
KR870018_Aurora_borealis_virus_strain_ABV_2
MH483056_S2_like_reptarenavirus_isolate_F18_5_4
KP071474_Reptarenavirus_Boa_dumerili_California_snake27_2009
KX527580_University_of_Giessen_virus_clone_S6_like_S131157
KP071502_Reptarenavirus_Boa_constrictor_California_snake22_2013
KP071578_Reptarenavirus_Boa_constrictor_Florida_snake26_2011
KP071509_Reptarenavirus_Boa_constrictor_California_snake35_2013
MH483067_S5_like_reptarenavirus_isolate_S14_0591_4
MH483088_S7_like_reptarenavirus_isolate_S17_172_7
MH503957_S10_like_virus_strain_S17_252_7
KP071473_Reptarenavirus_Boa_dumerili_California_snake27_2009
KP071501_Reptarenavirus_Boa_constrictor_California_snake22_2013_(S6B)
MN567042_ArBV_1_Snake #1
MN567052_ArBV_1_Snake #2
MN567058_ArBV_1_Snake #3
KP071559_Reptarenavirus_Boa_constrictor_Florida_snake34_1997_(S11)
NC_023766_University_of_Helsinki_virus_strain_UHV_1
MN567064_PAV_1_Snake #4
KR870016_University_of_Helsinki_virus_strain_UHV_2
KP071532_Reptarenavirus_Corallus_annulatus_California_snake1_2009
M20869_LCMV
KR870017_HISV-1
0.3

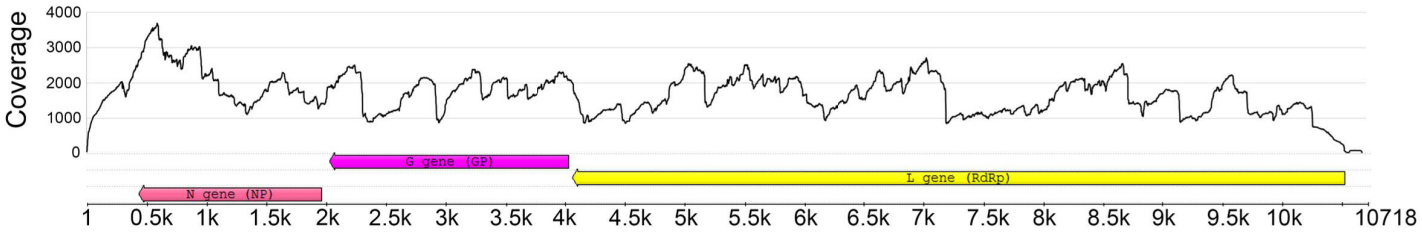

B
KP071501_Reptarenavirus_Boa_constrictor_California_snake22_2013_(S6B)
KP071502_Reptarenavirus_Boa_constrictor_California_snake22_2013
KX527580_University_of_Giessen_virus_clone_S6_like_S131157
KP071630_Reptarenavirus_Boa_constrictor_California_snake7_2008
UHV_est_10kb
KR870018_Aurora_borealis_virus_strain_ABV_2
MH483056_S2_like_reptarenavirus_isolate_F18_5_4
KP071474_Reptarenavirus_Boa_dumerili_California_snake27_2009
NC_039007_Tavallinen_suomalainen_mies_virus_clone_TSMV_2_S_F15133
MH483067_S5_like_reptarenavirus_isolate_S14_0591_4
KP071509_Reptarenavirus_Boa_constrictor_California_snake35_2013
KP071559_Reptarenavirus_Boa_constrictor_Florida_snake34_1997_(S11)
MN567042_ArBV_Snake #1
MN567052_ArBV_Snake #2
MN567058_ArBV_Snake #3
KR870016_University_of_Helsinki_virus_strain_UHV_2
NC_023761_Boa_arenavirus_NL_isolate_3
NC_023766_University_of_Helsinki_virus_strain_UHV_1
MN567064_PAV_1_Snake #4
KP071473_Reptarenavirus_Boa_dumerili_California_snake27_2009
KP071578_Reptarenavirus_Boa_constrictor_Florida_snake26_2011
MH483088_S7_like_reptarenavirus_isolate_S17_172_7
MH503957_S10_like_virus_strain_S17_252_7
KP071532_Reptarenavirus_Corallus_annulatus_California_snake1_2009
M20869_LCMV
KR870017_HISV-1
0.3


A
NC_043443_Haartman_Institute_snake_virus_isolate_1
MH483042_Haartman_Institute_snake_virus_2_isolate_S17_252_2
MH778629_Dante_Muikkunen_virus_1_isolate_F18_5
MH483032_Veterinary_Pathology_Zurich_virus_1_isolate_S14_369_79_2
MH483044_Veterinary_Pathology_Zurich_virus_1_isolate_Sn36_2
MH483028_Old_schoolhouse_virus_1_isolate_Ger_pool_2
MH483030_Old_schoolhouse_virus_2_isolate_Ger_pool_4
MN567050_SPVV_1_L1of2_Snake #1
MN567056_AHeV_1_Snake #2
MN567062_AHeV_1_Snake #3
J04331_LCMV
JQ717263_Golden_Gate_virus
0.2


B
NC_043444_Haartman_Institute_snake_virus_isolate_1
MH483041_Haartman_Institute_snake_virus_2_isolate_S17-252_1
MH483026_Dante_Muikkunen_virus_1_isolate_F18-5_1
MH483031_Veterinary_Pathology_Zurich_virus_1_isolate_S14-369-79_1
MH483043_Veterinary_Pathology_Zurich_virus_2_isolate_Sn36_1
MH483027_Old_schoolhouse_virus_1_isolate_Ger-pool_1
MH483029_Old_schoolhouse_virus_2_isolate_Ger-pool_3
MN567048_SPVV-1_Snake #1
MN567055_AHeV-1_Snake #2
MN567061_AHeV-1_Snake #3
M20869_LCMV
NC_018483_Golden_Gate_virus
0.3


C
NC_043444_Haartman_Institute_snake_virus_isolate_1
MH483041_Haartman_Institute_snake_virus_2_isolate_S17-252_1
MH483026_Dante_Muikkunen_virus_1_isolate_F18-5_1
0,78
MH483027_Old_schoolhouse_virus_1_isolate_Ger-pool_1
MH483029_Old_schoolhouse_virus_2_isolate_Ger-pool_3
MH483031_Veterinary_Pathology_Zurich_virus_1_isolate_S14-369-79_1
MH483043_Veterinary_Pathology_Zurich_virus_2_isolate_Sn36_1
MN567048_SPVV-1_Snake #1
MN567055_AHeV-1_Snake #2
MN567061_AHeV-1_Snake #3
NC_018483_Golden_Gate_virus
M20869_LCMV
0.3

A



B

	ORF1 (RdRp, L gene)	ORF2 (Glycoprotein, G gene)	ORF3 (Nucleoprotein, N gene)
Protein size (amino acids, AA)	2144	660	503
Signal peptide	No	1-17	No
Transmembrane	2125-2142	542-563	No
Closest match by protein BLAST (identity at AA level)	L protein [Guangdong red-banded snake chuvirus-like virus], AVM87272.1 (48.56%)	putative glycoprotein [Guangdong red-banded snake chuvirus-like virus], AVM87273.1 (43.06%)	putative nucleoprotein [Guangdong red-banded snake chuvirus-like virus], AVM87274.1 (41.97%)
Domains identified by protein BLAST (domain, AA region, E-value)	Mononegavirales RNA dependent RNA polymerase (72-1032, 4.25E-60) Mononegavirales mRNA-capping region V (1038-1282, 2.03E-12) mRNA capping enzyme, paramyxovirus family (1615-1855, 8.69E-3)	None	None
Protein Information Resource Superfamily (PIRSF) matches (Accession no; AA region; E-value)	RNA-directed RNA polymerase, Paramyxoviridae type (PIRSF000830; 21-584, 594-1066, 1703-1985; 1.7e-24)	None	None
Pfam matches (Accession no; AA region; E-value)	Mononeg_RNA_pol (PF00946.19; 55-1013 and 1708-1734; 2.1e-73 and 0.22) Mononeg_mRNAcap (PF14318.6; 1038-1284; 4.1e-19)	None	Hypoxia Inducible Factor-1, HIF-1 (PF11413.8; 154-167; 3.8e-06) Arginine repressor, DNA binding domain (PF01316.21; 39-65 and 283-310; 0.79 and 6.4e-05)

C