

Distantly Related Rotaviruses in Common Shrews, Germany, 2004–2014

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We screened samples from common shrews (*Sorex araneus*) collected in Germany during 2004–2014 and identified 3 genetically divergent rotaviruses. Virus protein 6 sequence similarities to prototype rotaviruses were low (64.5% rotavirus A, 50.1% rotavirus C [tentative species K], 48.2% rotavirus H [tentative species L]). Shrew-associated rotaviruses might have zoonotic potential.

Rotaviruses are a major cause of diarrhea in young children, causing an estimated 215,000 deaths worldwide every year (1). These viruses are nonenveloped and have a genome consisting of 11 segments of double-stranded RNA (2); each segment codes for either 1 of the structural proteins, virus protein (VP) 1–7, or 1 or 2 of the nonstructural proteins (NSPs), NSP1–6. Rotaviruses are classified into species A–I or the tentative species J on the basis of the amino acid sequence similarity of the conserved structural protein VP6 and the conserved nucleotide sequence of the genome segment ends (3–5). For rotavirus A, further classification into genome segment-specific genotypes has been established (6). Rotaviruses can infect a wide diversity of animals, and zoonotic transmission of rotaviruses has been reported (7).

Shrews are small insectivorous mammals that have been previously identified as reservoirs for other pathogens (e.g., hantaviruses and *Leptospira* spp.) (8–10). In this investigation, we aimed to determine whether common shrews (*Sorex araneus*, order Eulipotyphla) are also a reservoir for rotaviruses and, if so, assess the genetic variability of the viruses found in this species.

The Study

During 2004–2014, small mammals were caught in different regions of Germany as part of local monitoring or

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pest control measures (9,10). From these animal collections, we acquired samples (intestine contents) collected from 49 common shrews (Figure 1). We combined these samples almost equally into 2 pools and performed RNA extraction followed by next-generation sequencing (NGS) using the Ion Torrent Personal Genome Machine system (ThermoFisher Scientific, <https://www.thermofisher.com>; Appendix, <https://wwwnc.cdc.gov/EID/article/25/12/19-1225-App1.pdf>). By applying the RIEMS data analysis pipeline (11), we identified 3 short contigs with low sequence similarities to rotavirus H in both pools. To identify the positive animals, we extracted RNA from individual samples and screened for rotavirus RNA using reverse transcription PCR (RT-PCR) with primers specific to 1 of the 3 rotavirus H contigs we previously obtained (Appendix Table 1). In total, 7 (15.2%) of 46 samples turned out to be positive for species H-like rotavirus (Table 1); 2 samples, KS/12/0644 and KS/11/2281, generated the strongest signal on ethidium bromide staining. We subjected these 2 samples to RNase and DNase treatment followed by RNA extraction and NGS using the NextSeq 500 sequencing system (Illumina, <https://www.illumina.com>); 8,576,782 read pairs for KS/12/0644 and 6,168,437 for KS/11/2281 were generated. After a RAMBO-K analysis (12) suggested a low abundance of highly deviant rotavirus sequences, we performed data analysis and contig assembly using a newly generated pipeline (Appendix). By this method, contig lengths were 164–3,017 nt, and we obtained 48 contigs with sequence similarities to rotavirus A, 17 with low sequence similarities to rotavirus C, and 23 with low sequence similarities to rotavirus H (Appendix Table 2). Because contigs of homologous genes from each of the 3 viruses were detected in these samples, we concluded 3 different rotaviruses were present in both.

We then performed RT-PCR with all samples using primers specific to the species A and species C-like rotavirus contigs from the previous analysis, and 21.7% (10/46) were positive for species A rotavirus and 10.9% (5/46) for species C-like rotavirus; rotavirus co-infections were also identified (Table 1). An analysis of the geographic distribution of shrew rotaviruses in Germany shows that species C-like rotaviruses were mainly located in the northeast and Southwest, species H-like rotaviruses mainly in the south,

and species A rotaviruses broadly throughout (Figure 1). At the monitoring site in Baden-Württemberg (southwest Germany), frequent detections of different rotaviruses and multiple co-infections were observed.

Despite several efforts, we could delineate only partial genomic sequences of rotaviruses from the NGS data. By application of primer ligation, rapid amplification of cDNA ends, and degenerated primer RT-PCR strategies, we acquired the complete open reading frames of VP1, VP6, and NSP5 of most viruses (Table 2). In addition, we

reamplified and sequenced the VP6 genes of all viruses by dideoxy chain-termination sequencing and confirmed the VP6 sequences obtained. Sequence analysis of these genes and in silico translation indicated 14.1%–65.6% amino acid sequence similarity to the respective proteins of other rotavirus species (Table 2). The Rotavirus Classification Working Group reviewed the sequences of the shrew rotavirus A genes in sample KS/11/2281 and designated the new genotypes R23 for VP1, I27 for VP6, and H23 for NSP5. The maximum amino acid sequence similarities to established

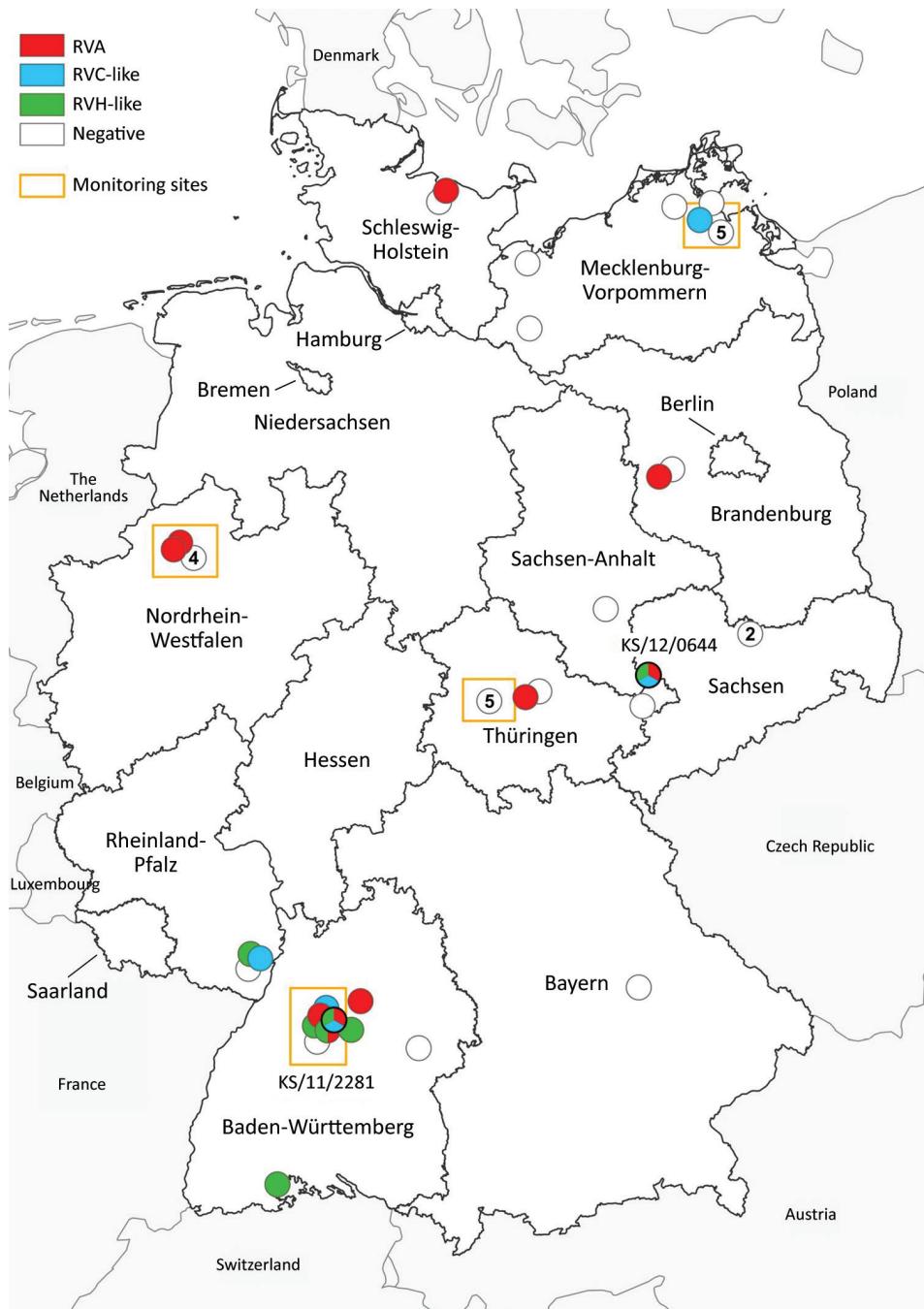


Figure 1. Distribution of common shrews (*Sorex araneus*) collected at monitoring sites (9) and additional sites (10) in Germany, 2004–2014, positive and negative for RVA, RVC-like, and RVH-like species by reverse transcription PCR. Numbers in white circles indicate the number of negative samples at that collection site; white circles without numbers indicate 1 negative sample at that site. Circles with multiple colors indicate animals with co-infections. The collection sites of the 2 samples analyzed in detail by next-generation sequencing (KS/12/0644 and KS/11/2281; tricolored circles) are indicated. RVA, rotavirus A; RVC, rotavirus C; RVH, rotavirus H.

Table 1. Rotavirus infections detected in common shrews (*Sorex araneus*) sampled in Germany, 2004–2014*

Virus species	Monoinfections	Co-infections with						Total infections
		RVA	RVC	RVH	RVA and RVC	RVC and RVH	RVA and RVH	
RVA	7/46 (15.2)	NA	0/46	1/46 (2.2)	NA	2/46 (4.3)	NA	10/46 (21.7)
RVC-like	3/46 (6.5)	0/46	NA	0/46	NA	NA	2/46 (4.3)	5/46 (10.9)
RVH-like	4/46 (4.3)	1/46 (2.2)	0/46	NA	2/46 (4.3)	NA	NA	7/46 (15.2)

*Samples from shrews were examined by reverse transcription PCRs specific for RVA, RVC-like, and RVH-like species. Values are no. positive/total (%). NA, not applicable; RVA, rotavirus A; RVC, rotavirus C; RVH, rotavirus H.

rotavirus type species of 50.1% for VP6 of species C–like rotavirus and 48.2% (species H) or 48.3% (species J) for VP6 of species H–like rotavirus suggest that these viruses should be classified as novel (tentative) rotavirus species (Table 2).

Phylogenetic analyses of the VP1, VP6, and NSP5 proteins indicate a consistent branching of shrew rotavirus A with other rotavirus A species and shrew species C–like rotavirus with other rotavirus C species. However, the species H–like rotavirus branches more variably within the rotavirus B–G–H–I–J cluster (Figure 2). A more detailed phylogenetic analysis of complete and additional partial genome segment nucleotide sequences of the shrew rotavirus A showed a basal branching at the cluster of other species A rotavirus sequences for most genes (Appendix Figure 1). In addition, phylogenetic analyses of partial amino acid sequences deduced from other genes of the shrew species C–like and H–like rotaviruses confirmed the relationship evident from analyses of the 3 completely sequenced open reading frames (Appendix Figure 2–4).

Shrews have been analyzed infrequently for rotaviruses. In 1 study, rotavirus antigen was detected in wild Chinese tree shrews (*Tupaia chinensis*, order Scandentia) (13), and in another study, species A rotaviruses not identical to those of our study (Appendix Figure 1) were identified in house shrews (*Suncus murinus*, order Eulipotyphla) from China (14). Here, a broader rotavirus screening of common shrew samples resulted in the identification of novel rotaviruses. The rotavirus detection rate of 10.9%–21.7% in the analyzed samples from animals from different regions of Germany suggests a wide circulation of rotaviruses in

shrews, although more samples should be analyzed in the future to clarify the association of rotaviruses with these animals. We also identified co-infections with >1 rotavirus, a regular finding in other animal host species (15).

The shrew rotavirus A sequences showed low similarities with other species A rotaviruses, resulting in the assignment of novel genotypes and suggesting a long-term separate evolution of these viruses in this shrew species. The 2 other rotaviruses identified showed even lower sequence similarities to the known rotavirus species. According to the cutoff value of 53% suggested for the differentiation of rotavirus species on the basis of the encoded VP6 amino acid sequence (5), both viruses should be considered new rotavirus species, which we tentatively designate rotavirus species K (for the rotavirus C–like species) and L (for the rotavirus H–like species). However, because their complete genome sequences have not been determined, a final classification of these viruses remains to be accomplished. At least the 5' and 3' termini of these rotavirus genome segments, which are conserved within known rotavirus species (2), should be determined. The low virus amounts in samples, restricted available sample volumes, presence of multiple viruses in single samples, and low sequence similarities for some virus genes might help explain the failure to generate complete genome sequences in our study.

Conclusions

We identified multiple, genetically divergent rotavirus species in common shrews in Germany. These animals should be further investigated as a potential reservoir for rotaviruses capable of infecting humans.

Table 2. Sequence similarities of deduced VP1, VP6, and NSP5 amino acid sequences of rotaviruses from common shrews (*Sorex araneus*), Germany, 2004–2014*

Comparator rotavirus species and strain	Rotavirus species type (shrew sample designation), protein								
	A (KS/11/2281)			C-like (KS/11/2281)			H-like (KS/12/0644)		
	VP1	VP6	NSP5	VP1†	VP6‡	NSP5	VP1	VP6	NSP5
A, SA11	65.6	64.5	47.6	48.6	40.2	24.5	26.8	20.3	17.6
B, WH-1	27.4	17.1	15.9	24.9	14.7	17.6	55.5	39.2	32.9
C, Bristol	48.0	42.8	24.6	63.2	50.1	31.5	25.8	20.0	16.8
D, 05V0059	51.8	39.8	18.2	48.3	36.1	17.8	25.7	20.3	14.5
F, 03V0568	57.5	34.1	20.2	48.5	32.3	22.8	26.0	15.2	17.4
G, 03V0567	26.4	19.1	12.4	25.9	17.1	17.1	56.1	40.7	34.8
H, J19	27.3	17.3	18.8	25.7	17.4	15.1	63.1	48.2	38.2
I, KE135/2012	26.2	17.6	14.1	26.0	13.8	15.9	59.0	44.5	33.3
J, BO4351/Ms/2014	27.0	18.1	15.2	25.2	14.5	14.1	63.0	48.3	43.9

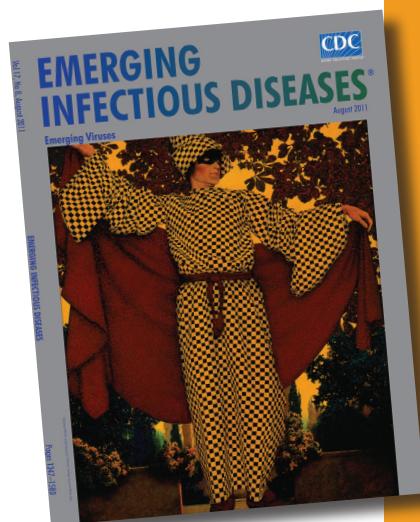
*Values are % sequence similarities. NSP, nonstructural protein; VP, virus protein.

†Incomplete at N terminus (≈70 aa residues missing) and C terminus (≈10 aa residues missing).

‡Incomplete at N terminus (≈40 aa residues missing).

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etymologia revisited

Rotavirus

[ro'tə-vi'rəs]

From the Latin *rota*, wheel, plus *virus*. After viewing the virus through an electron microscope in 1974, Flewett et al. suggested the name rotavirus on the basis of the pathogen's shape. The International Committee on Taxonomy of Viruses approved the name 4 years later.

Source: Dorland's illustrated medical dictionary. 31st edition. Philadelphia: Saunders, 2007; Flewett TH, Bryden AS, Davies H, Woode GN, Bridger JC, Derrick JM. Relation between viruses from acute gastroenteritis of children and newborn calves. *Lancet.* 1974;304:61–3. doi:10.1016/S0140-6736(74)91631-6; Matthews RE. Third report of the International Committee on Taxonomy of Viruses. Classification and nomenclature of viruses. *Intervirology.* 1979;12:129–296. doi:10.1159/000149081

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Appendix

Materials and Methods

Samples

Within a small mammal monitoring program, shrews were collected as bycatches during 2010–2013 at sites in 4 federal states (1). Additional shrews were collected between 2004 and 2014 in different regions of Germany (2). Collection of samples was done according to relevant legislation and by permission of the federal authorities (permits Regierungspräsidium Stuttgart 35–9185.82/0261, Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen 8.87–51.05.20.09.210, Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern 7221.3–030/09, Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz 22–2684–04–15–107/09) or were bycatches of the vole monitoring by local forestry institutions as part of their pest control measures. Trapping of the shrews was based on snap trapping, or the shrews were found dead in live traps.

The dissection followed a standard protocol including the collection of intestine samples. For this purpose, the frozen carcasses were thawed, intestinal contents (≈ 0.2 g) collected, transferred into fresh individual tubes and frozen again at -20°C until further analysis. In this investigation, 49 common shrews (*Sorex araneus*) were included.

Next-Generation Sequencing (NGS) Analysis of Pooled Samples

Initially, 2 pools were generated: 1 pool of 24 samples was generated by including 6 samples from each of the 4 monitoring sites. A second pool was generated by 25 samples from ongoing monitoring activities in 10 federal states of Germany. RNA was extracted from the pooled samples using the RNeasy Mini kit (QIAGEN, Hilden, Germany). Subsequently, the samples were processed as described (3). Briefly, double-stranded cDNA was generated using the cDNA-Synthesis System-Kit (Roche Life Science, Indianapolis, USA) and then transformed

into Ion Torrent compatible libraries. To this end, DNA was fragmented to a peak size of ≈ 500 bp with a M220 Focused-ultrasonicator (Covaris Inc., Brighton, UK). Then, fragment end-polishing and ligation of adapters (Ion Xpress Barcode Adapters; Life Technologies, Darmstadt, Germany) were performed with a GeneRead DNA Library L Core Kit (QIAGEN). After quality control (Agilent Bioanalyzer 2100 and High Sensitivity DNA Kit; both Agilent, Waldbronn, Germany) and quantification (KAPA Library Quantification Kit - Ion Torrent Personal Genome Machine (PGM) (Uni); Roche, Mannheim, Germany), libraries were pooled and sequenced with an Ion Torrent PGM in 400 bp run using HiQ reagents (Life Technologies, Darmstadt, Germany).

RT-PCR Screening for Specific Rotaviruses in Fecal Samples

A total of 46 individual samples of intestinal contents (3 of the initial 49 samples had been used up in other experiments) were diluted 1:5 in phosphate-buffered saline (PBS), and 100 μ l of the solution was subsequently subjected to nucleic acid extraction using the NucliSENS[®] easyMAG system (BioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Detection of rotaviruses was done by reverse transcription PCR (RT-PCR) using the One-Step RT-PCR kit (QIAGEN) with primers as described (Appendix Table 1) in a 2720 thermal cycler (Applied Biosystems). The thermal profile comprised 42°C for 30 min and 95°C for 15 min, followed by 40 cycles at 94°C for 30 s, 56°C for 30 s, and 74°C for 40 s, with a final incubation at 74°C for 5 min. PCR products were separated by electrophoresis on ethidium bromide-stained agarose gels.

NGS Analysis of Samples KS/12/0644 and KS/11/2281

A total of 100 μ l of each individual diluted fecal sample was clarified by centrifugation at 9,600 x g for 10 min. Thereafter, 70 μ l of the supernatant were mixed with 48.5 μ l nuclease-free water, 14 μ l 10x DNase buffer, 7 μ l TurboDNase (Invitrogen), and 0.5 μ l RNase A (QIAGEN). After incubation at 37°C for 1 h, RNA was extracted from the sample using the QIAamp viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions, but without adding carrier-RNA. The RNA was eluted in 60 μ l elution buffer (EB) and thereafter subjected to reverse transcription and random amplification using the WTA2 Whole Transcriptome Amplification Kit (Sigma, Deisenhofen, Germany). Briefly, 10 μ l of RNA preparation was mixed with 2.5 μ l Library Synthesis Solution and 4.1 μ l Nuclease-Free Water, heated at 95°C for 2 min, and immediately cooled to 18°C. Thereafter, 2.5 μ l Library Synthesis Buffer, 3.9 μ l

water, and 2.0 µl Library Synthesis Enzyme were added and incubated at 18°C for 10 min, 25°C for 10 min, 37°C for 30 min, 42°C for 10 min, and 72°C for 20 min, before cooling at 4°C. The following PCR was performed according to the manufacturer's instructions with 10 µl mixture from before in a total volume of 75 µl. The reaction mix was incubated at 94°C for 2 min and 17 cycles at 94°C for 30 sec and 70°C for 5 min each were performed. For NGS, the DNA libraries were generated from the PCR products with the TruSeq® Nano DNA Library Prep Kit (Illumina) and paired-end sequenced with 2 × 151 cycles using the NextSeq 500 sequencing system (Illumina).

The resulting 8,576,782 and 6,168,437 read pairs for samples KS/12/0644 and KS/11/2281 were subjected to trimming with fastp v0.14.1 with default parameters (4), yielding 6,498,311 and 4,878,954 read pairs, respectively. For a fast test for the presence of rotavirus reads with low similarity to known references, we classified rotavirus reads using RAMBO-K v1.21 (5). As a SPAdes v3.12.0 assembly (6) of the selected reads yielded only incomplete rotavirus genomes, we then assembled all trimmed reads using metaSPAdes (6). Resulting contigs were searched in the complete NCBI nr database using BLASTX v.2.6.0 with wordsize 4 (7). Contigs with hits to any rotavirus sequence were selected as putative rotavirus sequences and subjected to further manual analysis (see below).

PCR, FLAC, and RACE for Completion of Segment Sequences

First trials to complete the 5'- and 3'-ends of the genome segments were done by full-length amplification of cDNA (FLAC) as described (8). Further trials were done by rapid amplification of cDNA ends (RACE) using the 5' RACE System Kit (Invitrogen GmbH, Karlsruhe, Germany) as described (9). In both cases, specific primers delineated from NGS-derived sequences were used. In an additional set of experiments, specific RT-PCRs with these primers and those derived from an alignment of segment ends of other available rotavirus sequences were performed to complete the open reading frames for virus protein (VP) 1, VP6, and nonstructural protein 5 (NSP5). The RT-PCR was performed as described above for detection of rotaviruses; however, the cycling conditions were adapted to primer sequences and product lengths. Products with the expected lengths were purified using the QiaQuick DNA Purification Kit (QIAGEN) and subjected to dideoxy chain termination sequencing by a commercial supplier (Eurofins, Ebersberg, Germany).

Sequence Data Analysis

The NGS contigs were manually inspected using the SeqBuilder module of the DNASTAR software package (Lasergene, Madison, WI, USA) and flanking primer sequences were removed. Sequences were assembled from these NGS contigs and sequences from PCR, FLAC, or RACE products using the same software. The sequences were submitted to the GenBank database and accession numbers MN307962–MN207992 were assigned (also indicated on the branches of the phylogenetic trees). Sequences of the rotavirus type species and the rotavirus A genotype reference strains were used for alignments and phylogenetic comparisons. Nucleotide sequences were used for genotyping and assessment of phylogenetic relationship to other rotavirus A strains in the case of shrew rotavirus A sequences. As only very low nucleotide sequence similarities were evident for the species C-like and H-like rotaviruses of shrews compared with the reference strains, the deduced amino sequences were used, which showed a higher degree of sequence conservation. The phylogenetic trees were constructed using a neighbor-joining method implemented in the MEGALIGN module of the DNASTAR software package (Lasergene), and bootstrap analysis with 1000 trials and 111 random seeds was performed. Genotyping of rotavirus A genome segments was done as described (10). According to this protocol, partial sequences can only be assigned to an existing genotype if they have a minimum length of 500 nt and cover more than 50% of the open reading frame. In case they are only distantly related to the established genotypes, the complete sequence of the open reading frame has to be submitted to the Rotavirus Classification Working Group for genotype assignment, which was done for the VP1-, VP6- and NSP5-encoding genome segments of the shrew rotavirus A.

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Appendix Table 1. Primers used for detection of RVA, RVC-like and RVH-like viruses in shrews*

Virus	Gene	Designation	Sequence (5'-3')	Product length, bp
RVA	VP1	S-RVA-s	CGTTTCACGTAGGATTCAGGAA	225
		S-RVA-as	CTCCATTTGACGCTGATGACAT	
RVC-like	VP1	S-RVC-like-s	AACTGTGTCAAATGACGTCACA	218
		S-RVC-like-as	GAAGTGTTCGATCGCACGATT	
RVH-like	VP3	RVH-like-s	CCAGACAACCTACTCATTCTGTTG	207
		RVH-like-as	TTGTGTGTGGTTCCTTTACCT	

*RVA, rotavirus A; RVC, rotavirus C; RVH, rotavirus H; VP, virus protein; bp, base pairs.

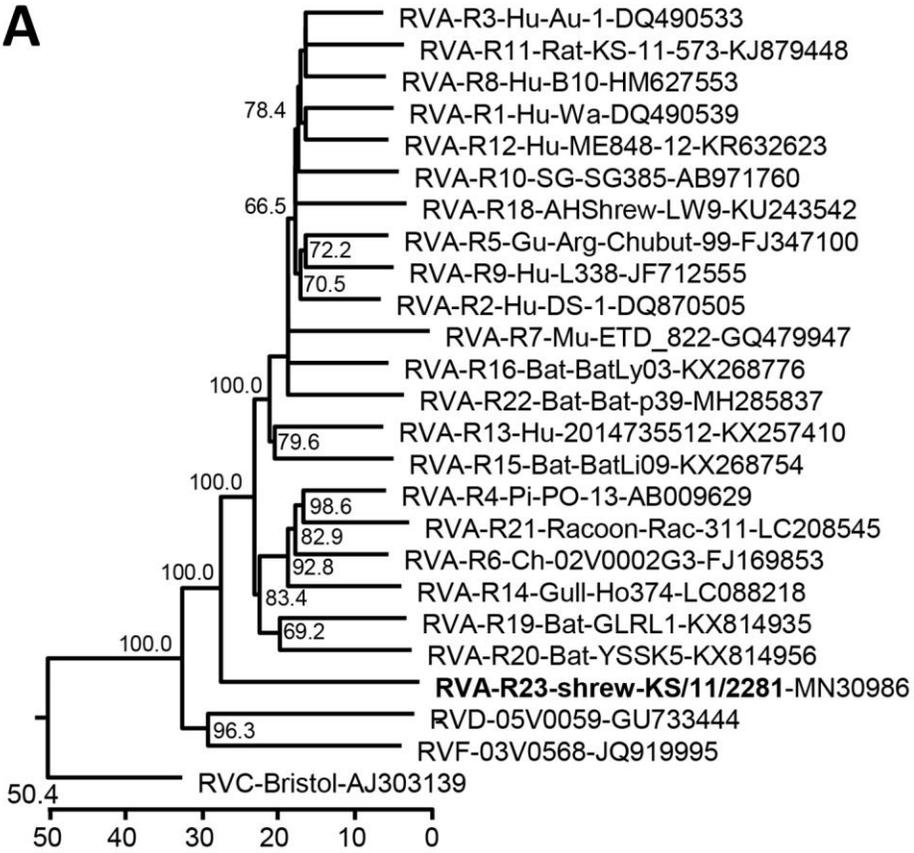
Appendix Table 2. Contig and read numbers and lengths generated by NGS of samples KS/12/0644 and KS/11/2281*

Category	KS/12/0644			KS/11/2281		
	RVA	RVC-like	RVH-like	RVA	RVC-like	RVH-like
Contig number	23	7	18	25	10	5
Contig lengths	200–1135	193–667	374–2584	164–821	636–3017	264–506
Read coverage	1.6–11.9	1.1–9.3	3.8–22.4	1.1–3.8	4.6–19.6	1.3–1.9
Total rotavirus read number		3597			2389	
Total read number†		8,576,782			6,168,437	

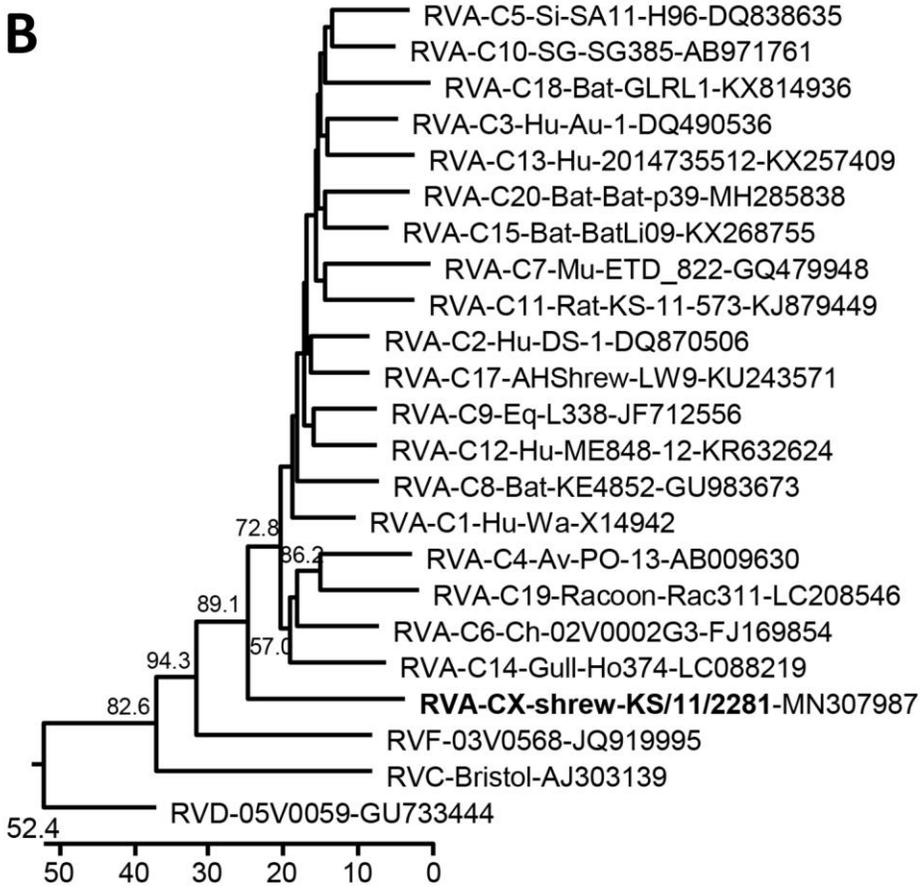
*NGS, next-generation sequencing; RVA, rotavirus A; RVC, rotavirus C; RVH, rotavirus H.

†number of all generated reads including rotavirus reads and non-rotavirus reads from the sample.

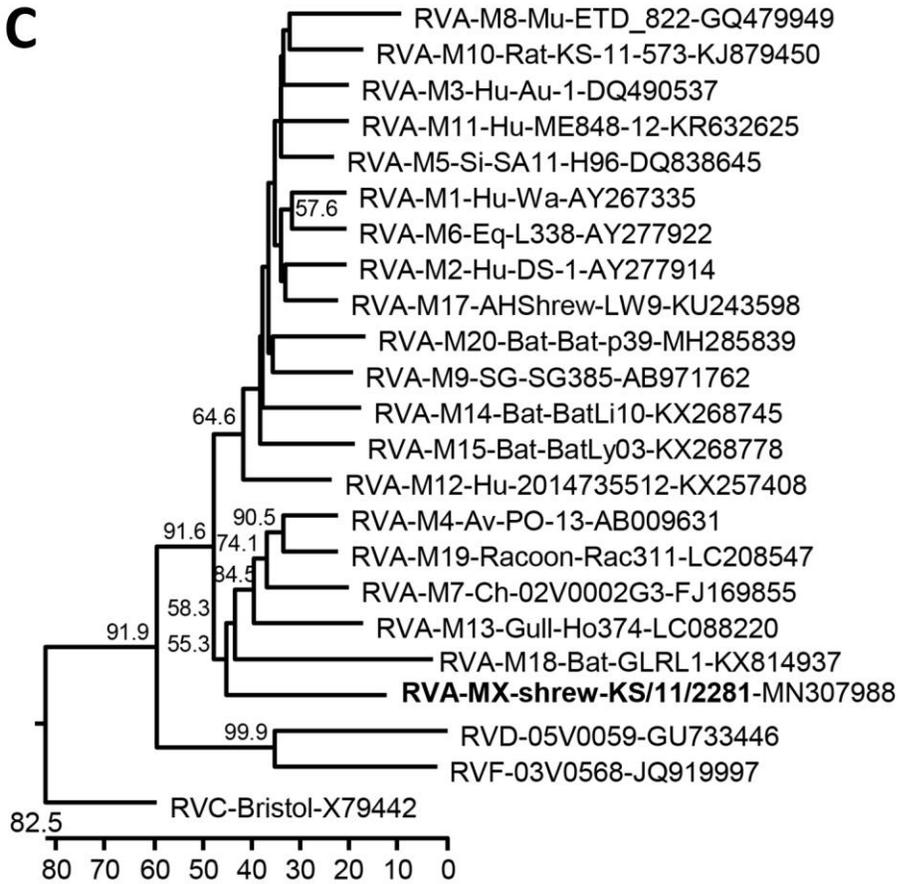
A



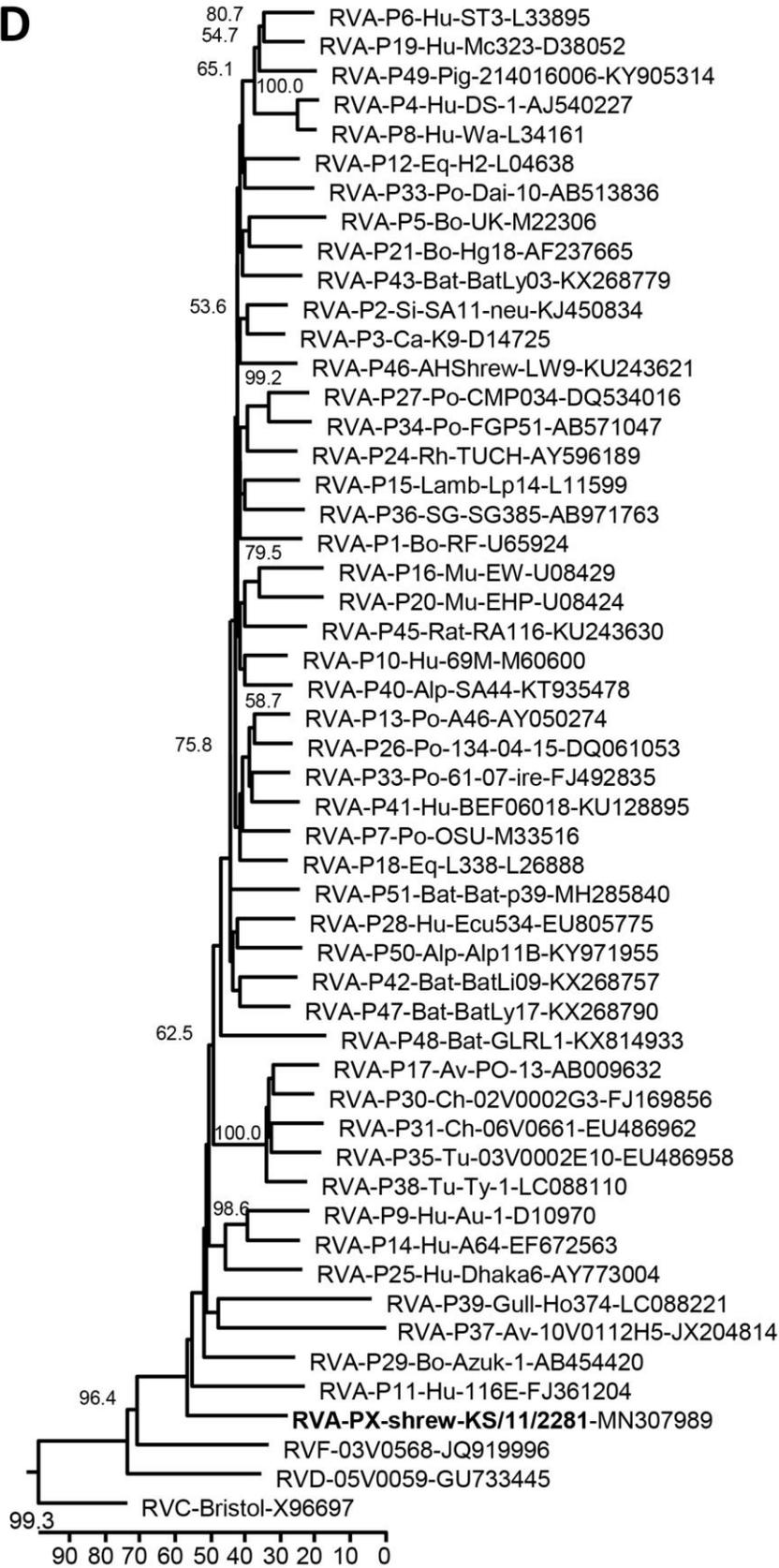
B



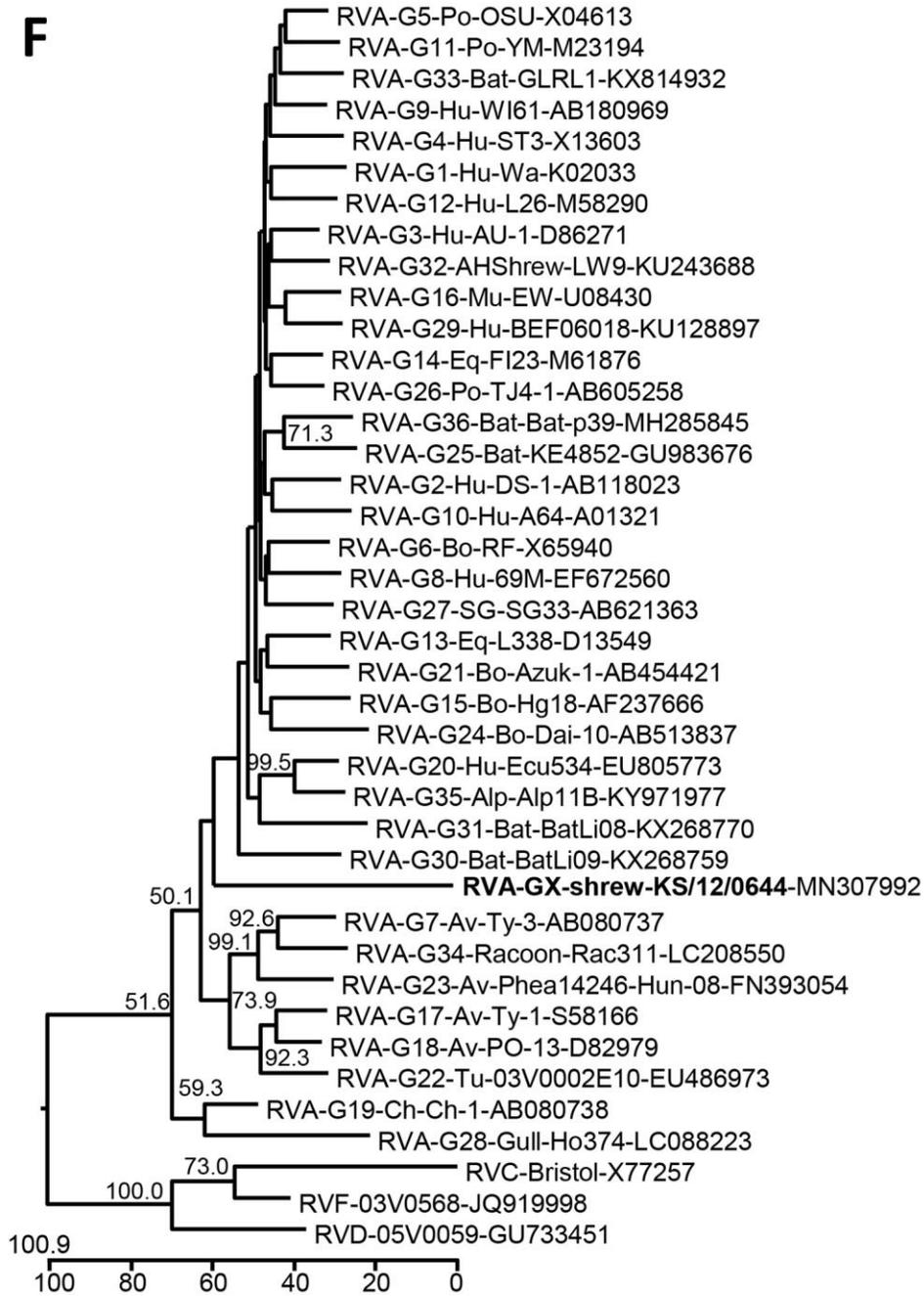
C



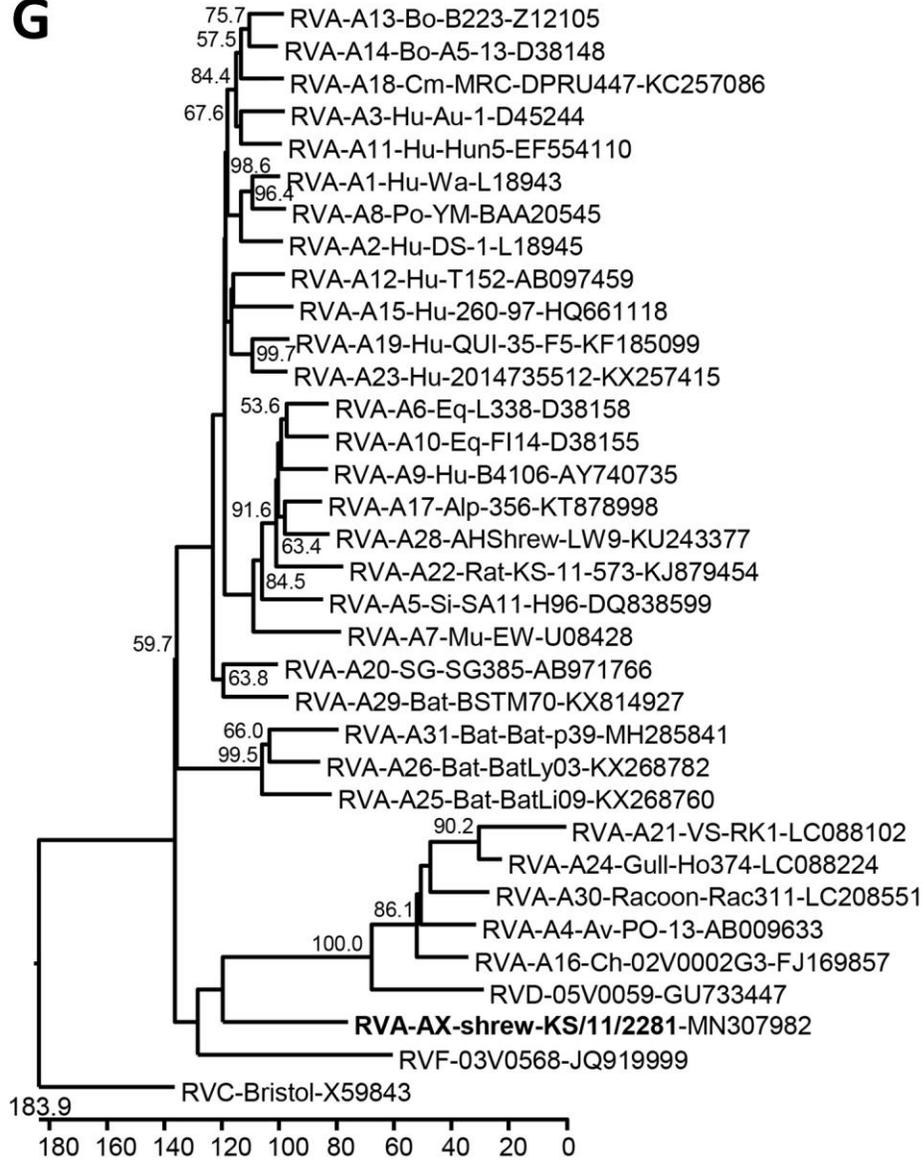
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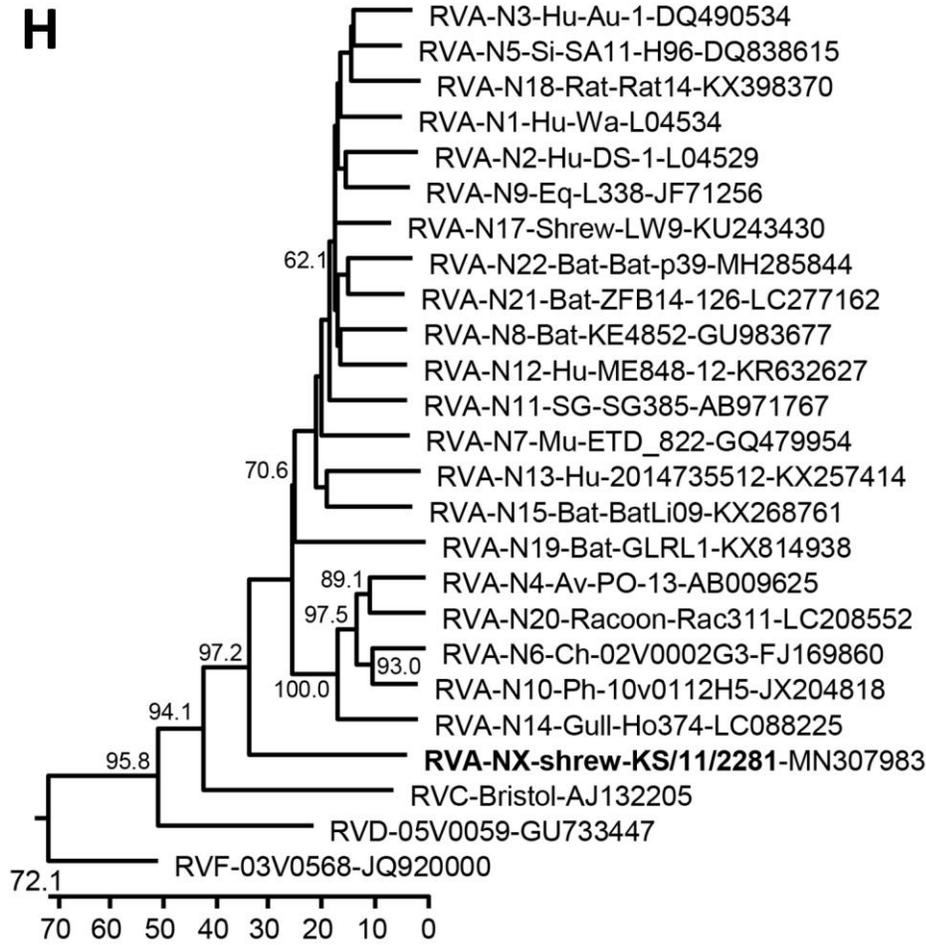
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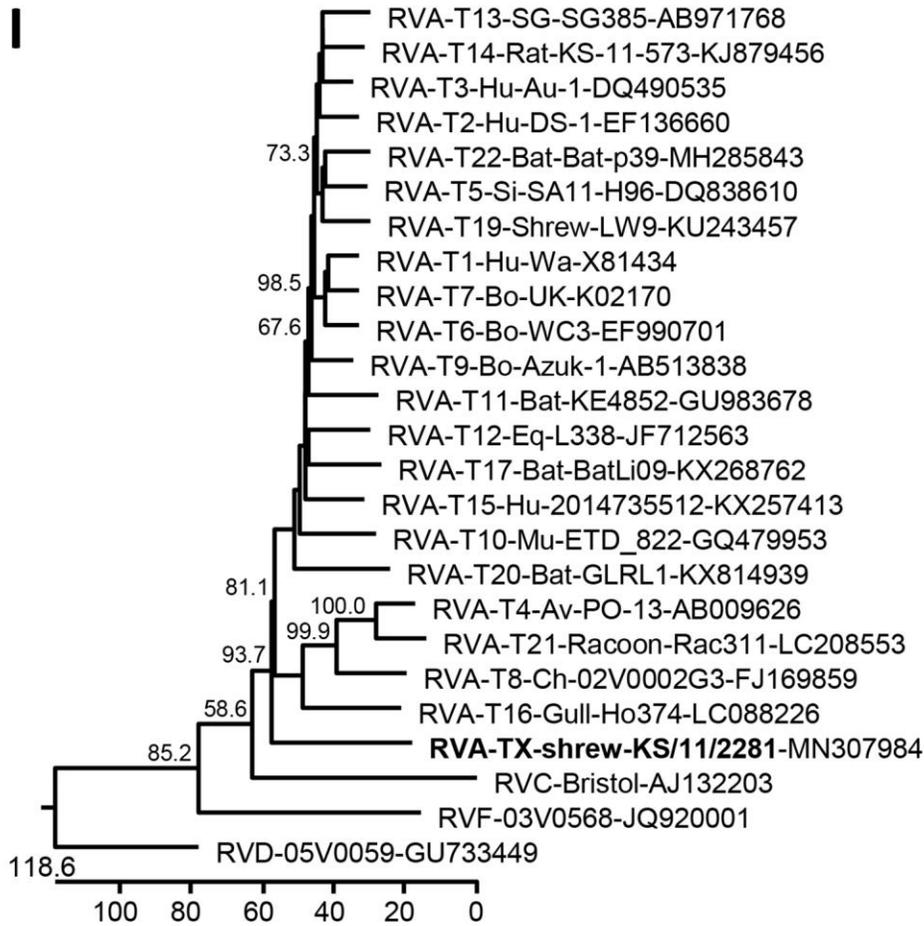


G

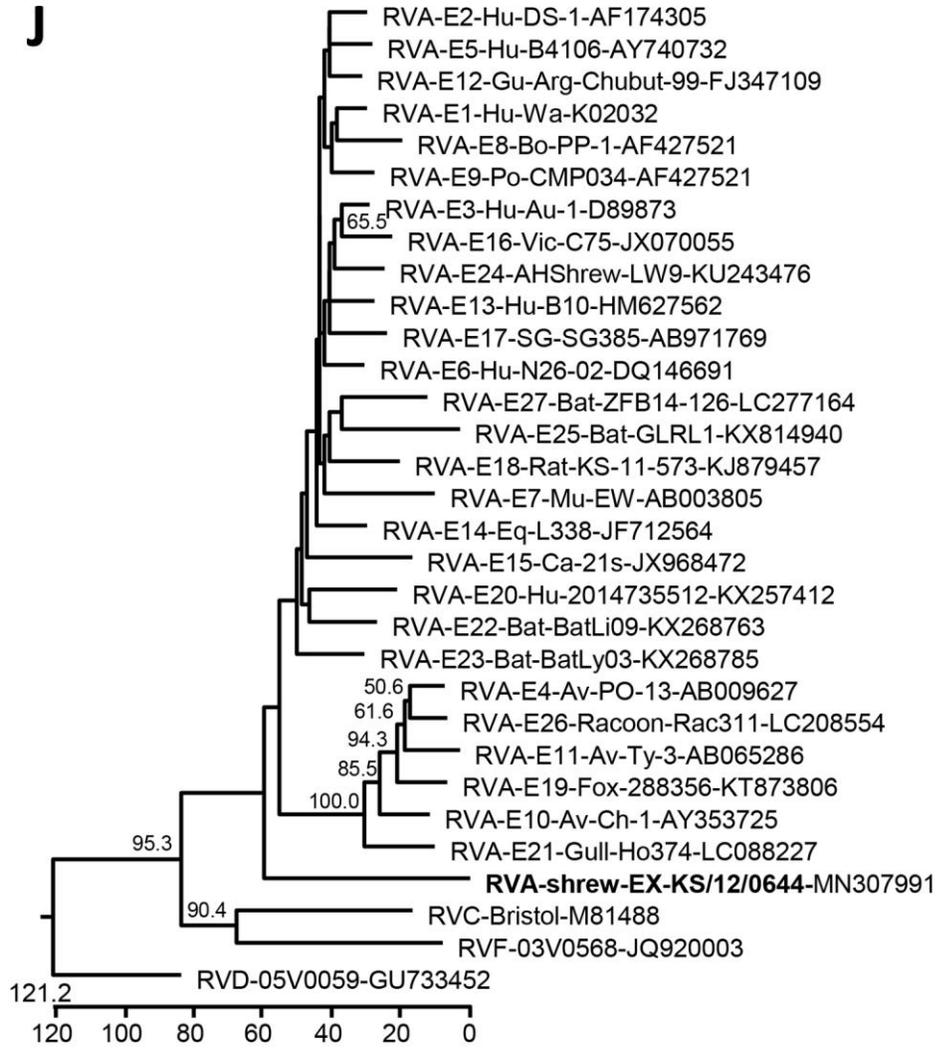


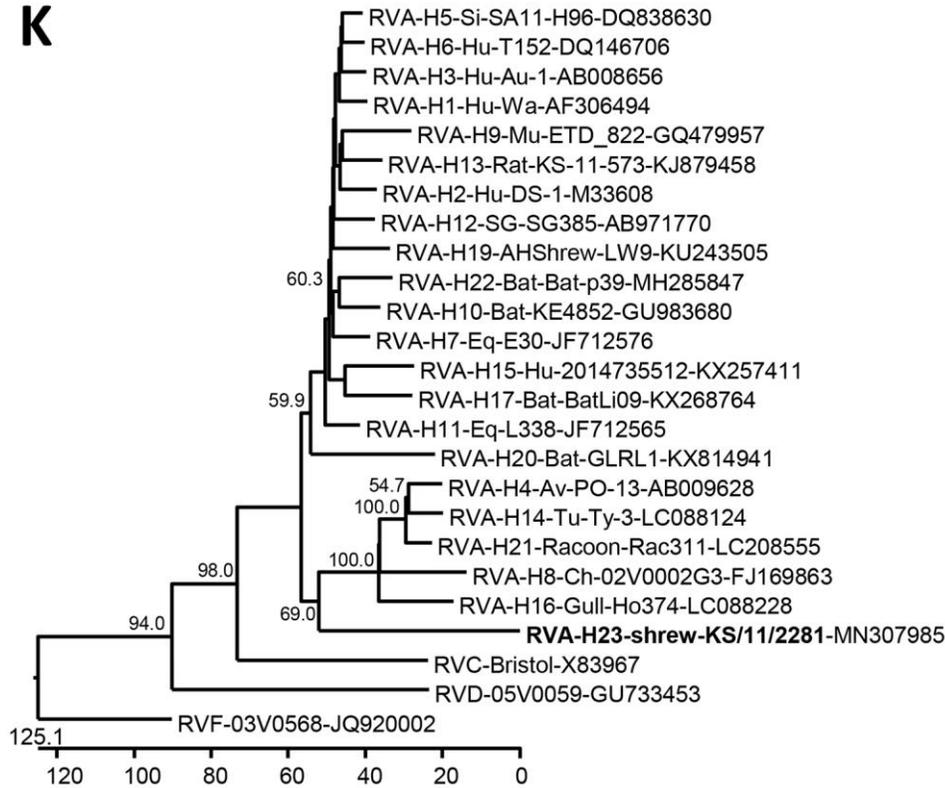
H



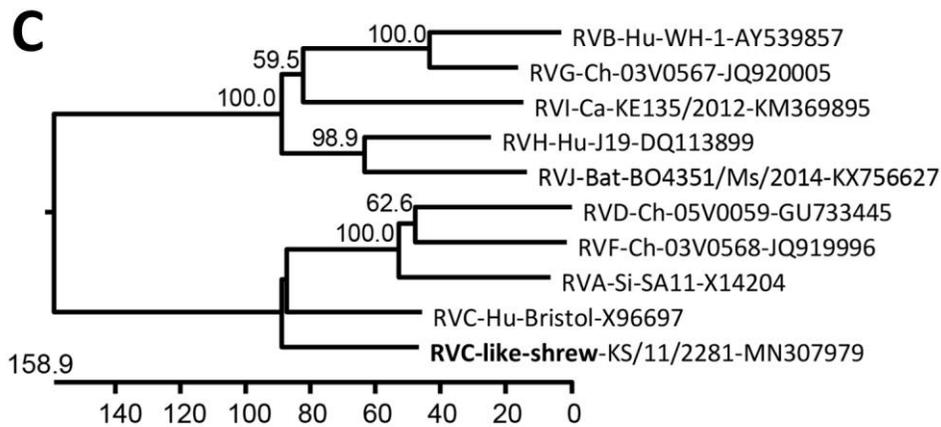
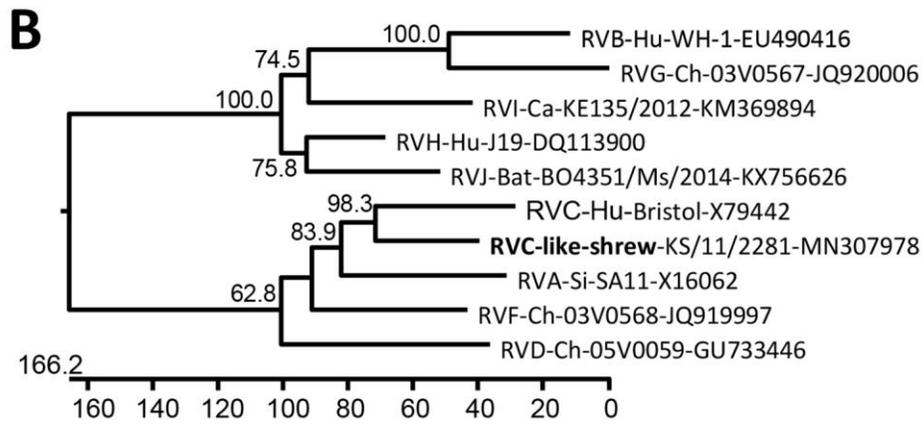
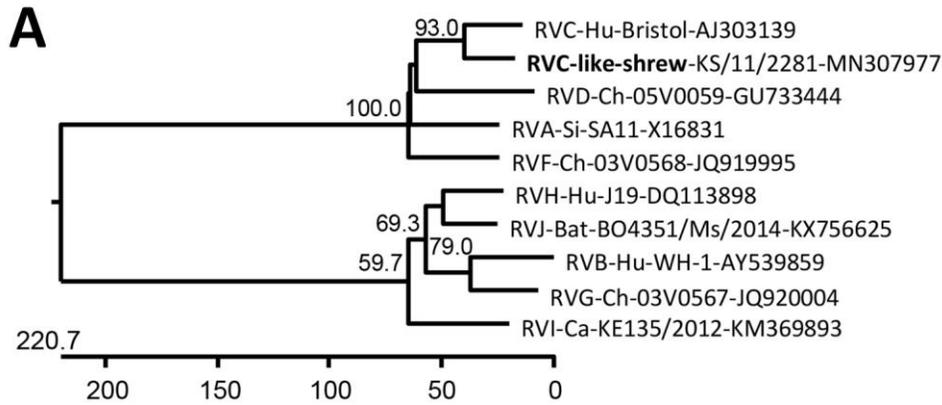


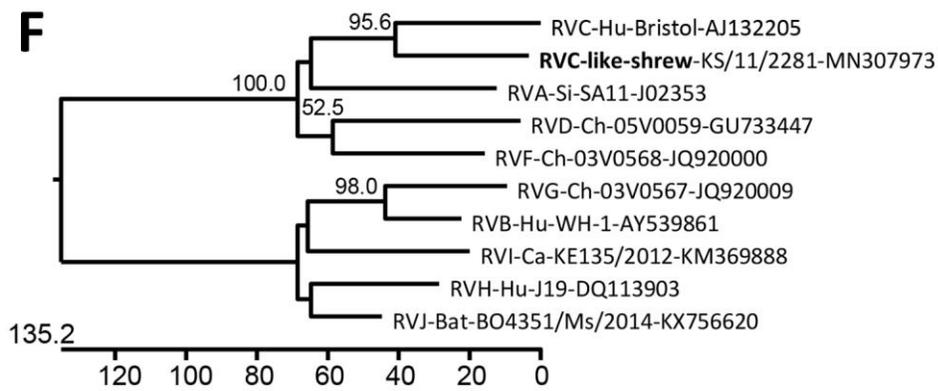
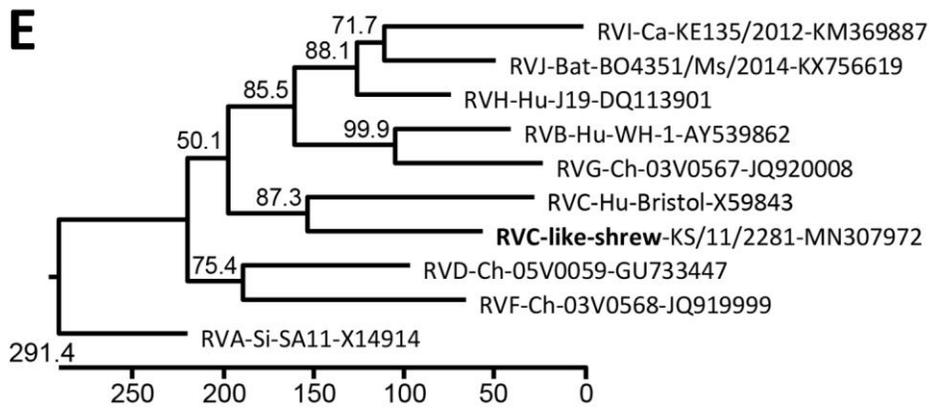
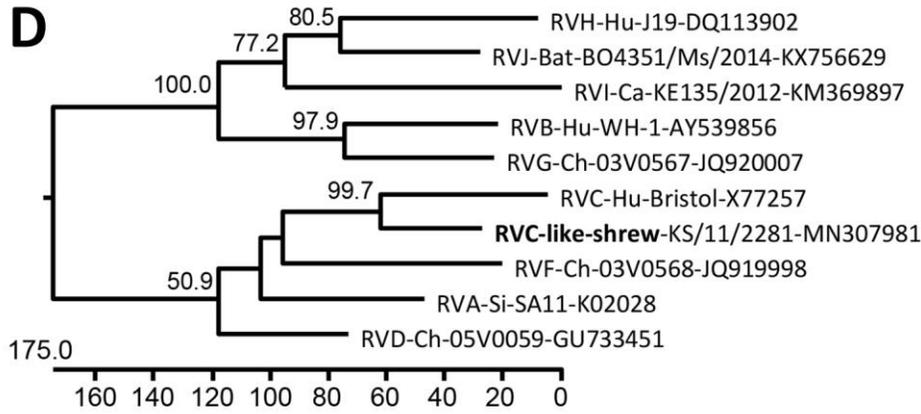
J

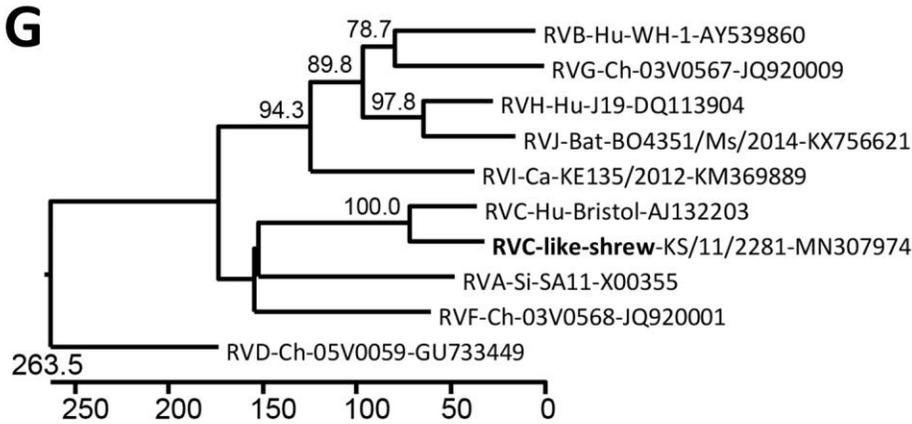


K

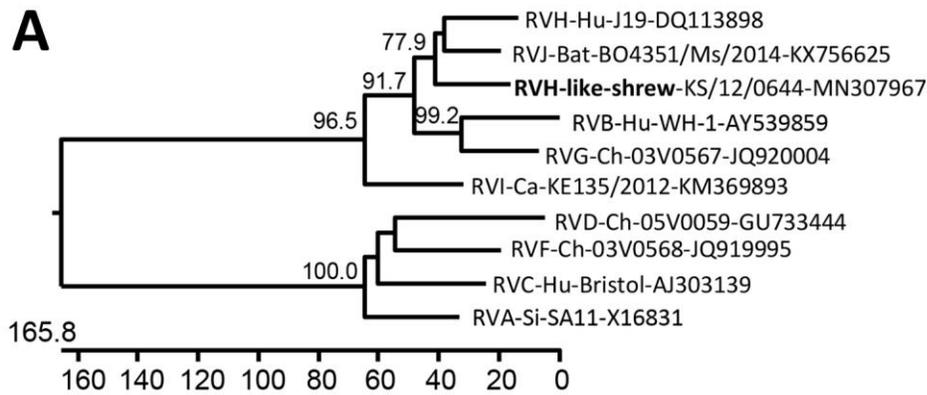
Appendix Figure 1. Phylogenetic relationship of the shrew rotavirus A (RVA) with genotype reference strains based on complete or partial nucleotide sequences of the genome segments encoding virus protein (VP) 1 (A), VP2 (B), VP3 (C), VP4 (D), VP6 (E), VP7 (F), nonstructural protein (NSP) 1 (G), NSP2 (H), NSP3 (I), NSP4 (J), and NSP5 (K). The rotavirus genotype, host (for abbreviations see below), strain designation and GenBank accession numbers are indicated at the branches of the tree. Rotavirus C (RVC), D (RVD), and F (RVF) strains are included as outgroups. The shrew virus from this study is given in bold letters. The tree was constructed using a neighbor-joining method implemented in the MEGALIGN module of DNASTAR software package (Lasergene) and a bootstrap analysis with 1000 trials and 111 random seeds. Bootstrap values of >50% are shown. The trees are scaled in nucleotide substitutions per 100 residues. Alp, alpaca; Av, avian; Bo, bovine; Ca, canine; Ch, chicken; Cm, camel; Eq, equine; Gu, guanaco; Hu, human; Mu, murine; Ph, pheasant; Pi, pigeon; Po, porcine; Ra, rabbit; Rh, rhesus monkey; RVA, rotavirus A; RVC, rotavirus C; RVD, rotavirus D; RVF, rotavirus F; SG, sugar glider; AHSshrew, Asian house shrew; shrew, common shrew from this study; Si, simian; Tu, turkey; Vic, vicuna.

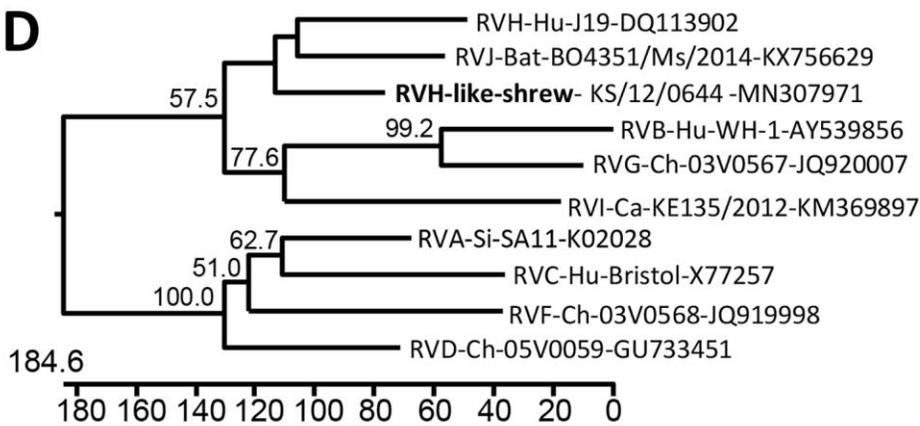
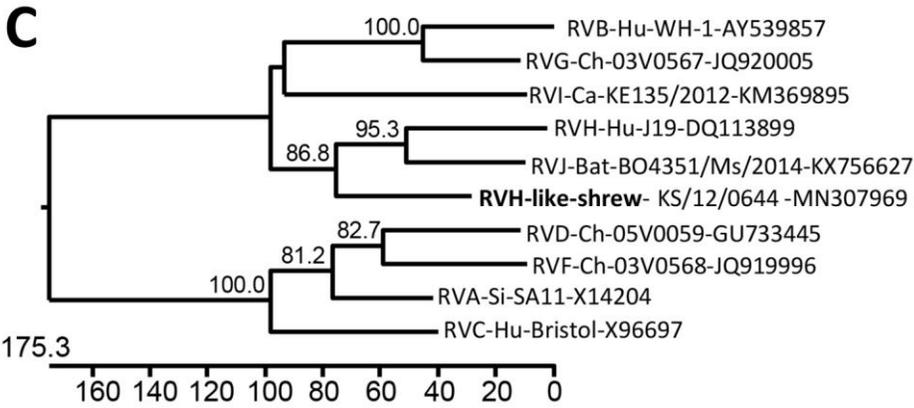
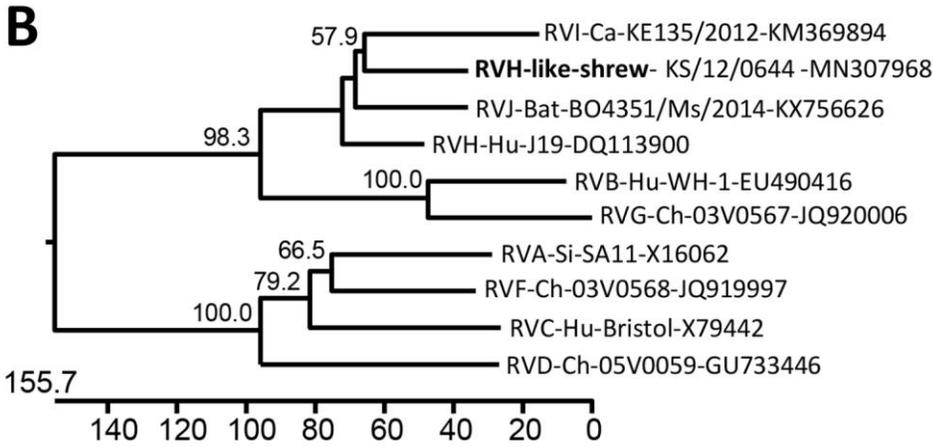


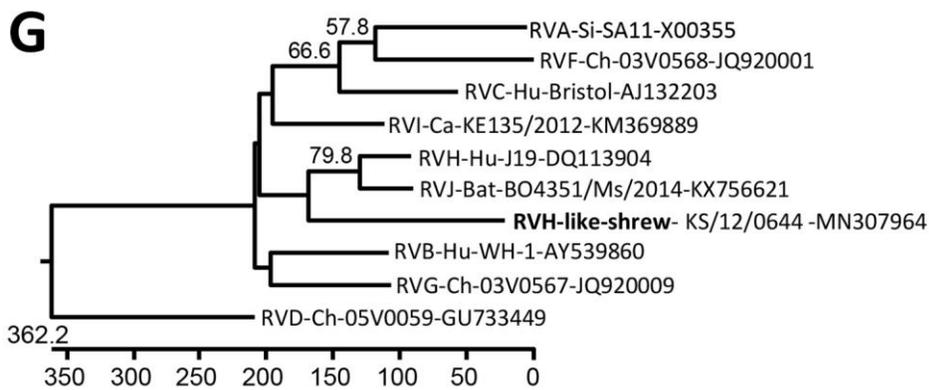
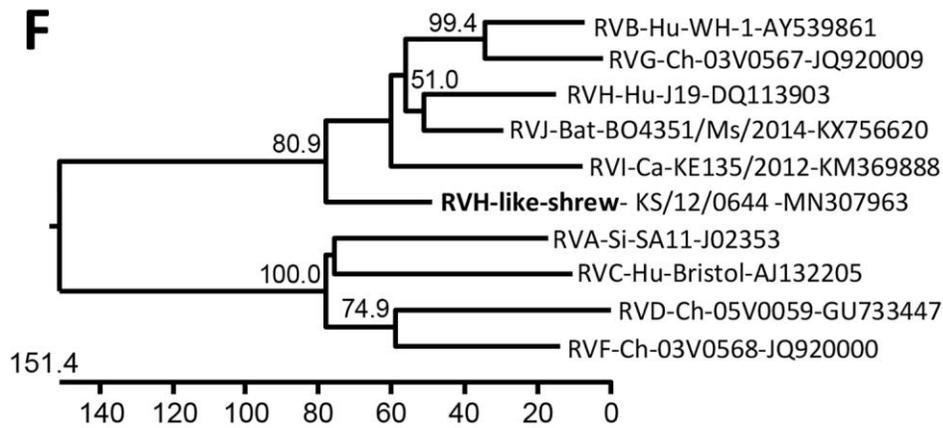
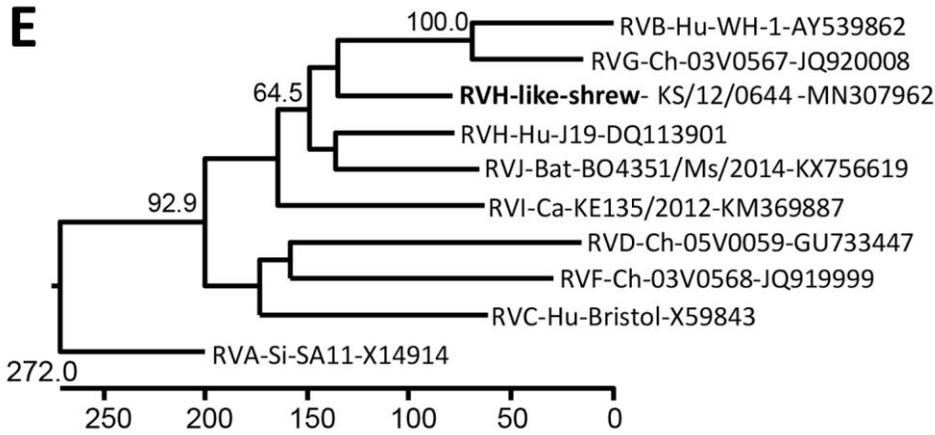




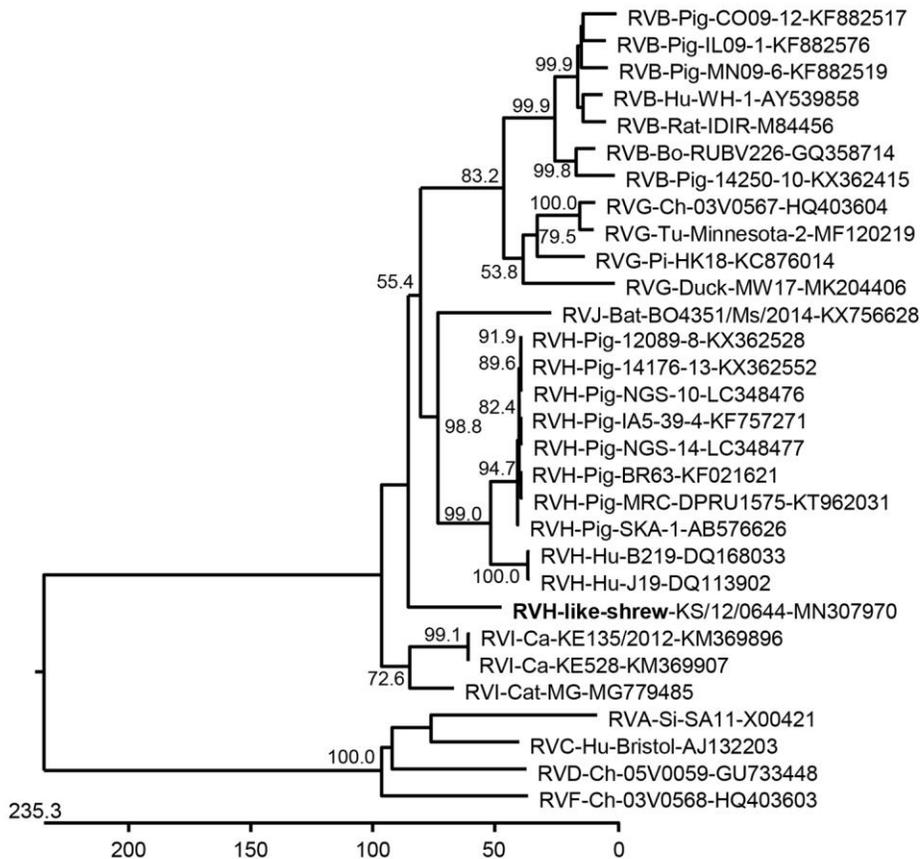
Appendix Figure 2. Phylogenetic relationship of the shrew species C-like rotavirus with the rotavirus A-J type species based on the deduced amino acid sequences of partial virus protein (VP) 2 (A), VP3 (B), VP4 (C), VP7 (D), nonstructural protein (NSP) 1 (E), NSP2 (F), and NSP3 (G). The rotavirus species (RVA-RVJ), host (for abbreviations see below), strain designation, and GenBank accession numbers are indicated at the branches of the tree. The shrew virus is given in bold letters. The tree was constructed using a neighbor-joining method implemented in the MEGALIGN module of DNASTAR software package (Lasergene) and a bootstrap analysis with 1000 trials and 111 random seeds. Bootstrap values of >50% are shown. The trees are scaled in amino acid substitutions per 100 residues. aa, amino acid residues; Ca, canine; Ch, chicken; Hu, human; RVA, rotavirus A; RVB, rotavirus B; RVC, rotavirus C; RVD, rotavirus D; RVF, rotavirus F; RVG, rotavirus G; RVH, rotavirus H; RVI, rotavirus I; RVJ, rotavirus J; Si, simian.







Appendix Figure 3. Phylogenetic relationship of the shrew species H-like rotavirus with the rotavirus A-J type species based on the deduced amino acid sequences of partial or complete virus protein (VP) 2 (A), VP3 (B), VP4 (C), VP7 (D), nonstructural protein (NSP) 1 (E), NSP2 (F), and NSP3 (G). The rotavirus species (RVA-RVJ), host (for abbreviations see below), strain designation, and GenBank accession numbers are indicated at the branches of the tree. The shrew virus is given in bold letters. The tree was constructed using a neighbor-joining method implemented in the MEGALIGN module of DNASTAR software package (Lasergene) and a bootstrap analysis with 1000 trials and 111 random seeds. Bootstrap values of >50% are shown. The trees are scaled in amino acid substitutions per 100 residues. aa, amino acid residues; Ca, canine; Ch, chicken; Hu, human; RVA, rotavirus A; RVB, rotavirus B; RVC, rotavirus C; RVD, rotavirus D; RVF, rotavirus F; RVG, rotavirus G; RVH, rotavirus H; RVI, rotavirus I; RVJ, rotavirus J; Si, simian.



Appendix Figure 4. Phylogenetic relationship of the shrew species H-like rotavirus within the rotavirus B/G/H/I/J cluster. Type species of rotavirus A, C, D, and F are included as outgroup. The complete deduced amino acid sequences of VP6 were used for construction of a phylogenetic tree using a neighbor-joining method implemented in the MEGALIGN module of DNASTAR software package (Lasergene) and a bootstrap analysis with 1000 trials and 111 random seeds. Bootstrap values of >50%

are shown. The tree is scaled in amino acid substitutions per 100 residues. The rotavirus species (RVA-RVJ), host (for abbreviations see below), strain designation, and GenBank accession numbers are indicated at the branches of the tree. The shrew virus is given in bold letters. Bo, bovine; Ca, canine; Ch, chicken; Hu, human; Pi, pigeon; RVA, rotavirus A; RVB, rotavirus B; RVC, rotavirus C; RVD, rotavirus D; RVF, rotavirus F; RVG, rotavirus G; RVH, rotavirus H; RVI, rotavirus I; RVJ, rotavirus J; Si, simian; Tu, turkey.