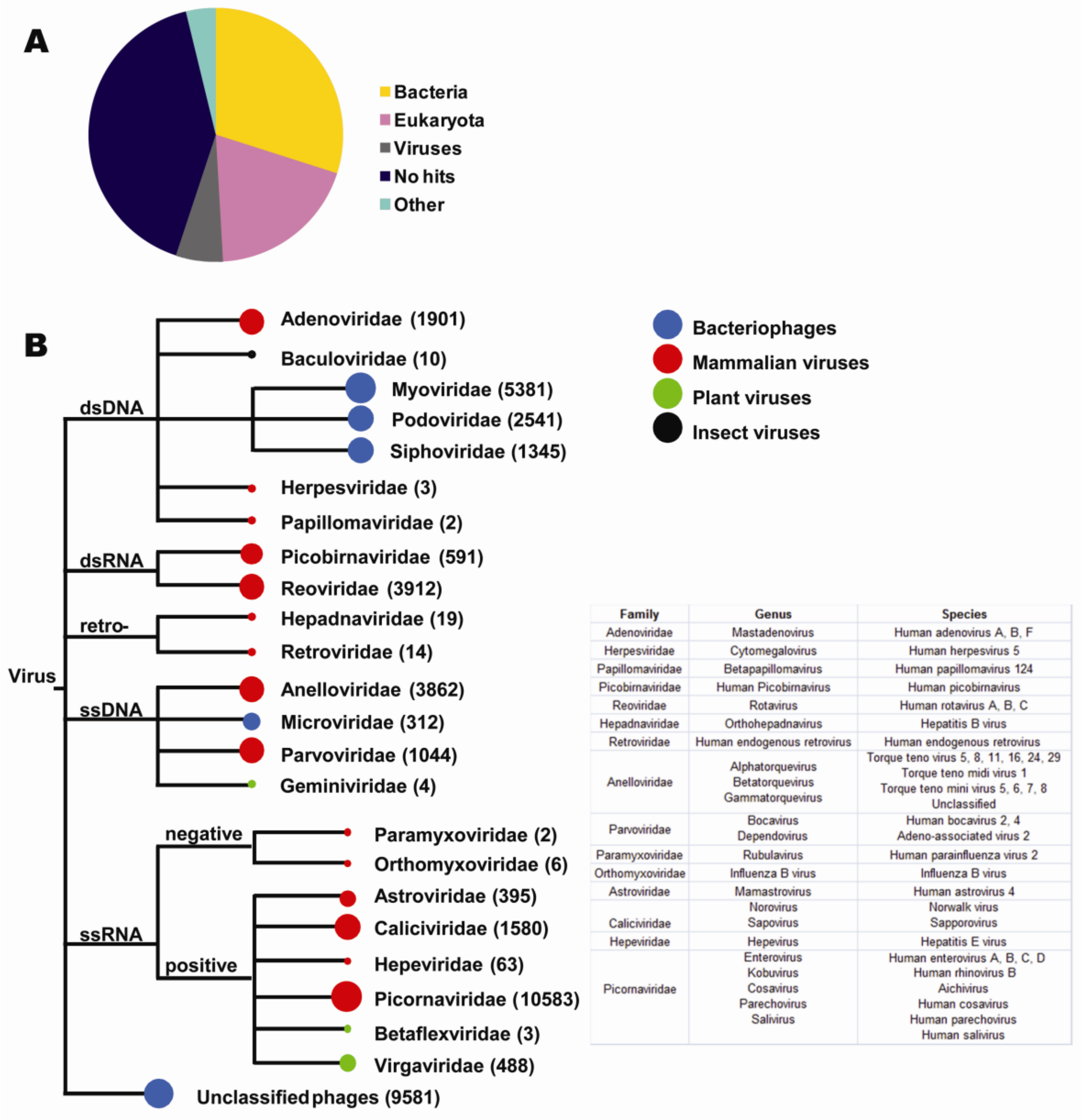
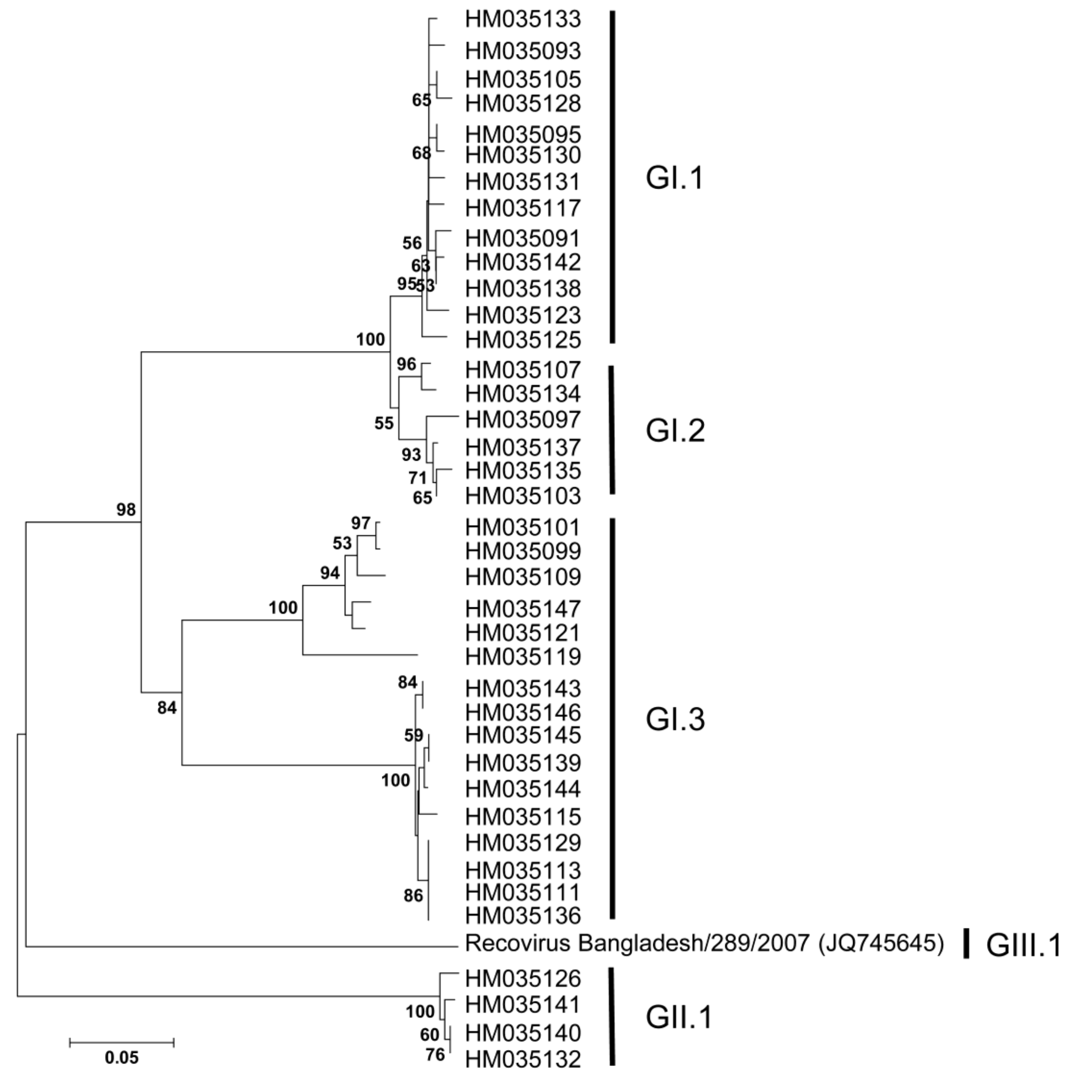
Detection of Human Calicivirus in Novel Recovirus Genogroup, Bangladesh

Technical Appendix



Family	Genus	Species
Adenoviridae	Mastadenovirus	Human adenovirus A, B, F
Herpesviridae	Cytomegalovirus	Human herpesvirus 5
Papillomaviridae	Betapapillomavirus	Human papillomavirus 124
Picobirnaviridae	Human Picobirnavirus	Human picobirnavirus
Reoviridae	Rotavirus	Human rotavirus A, B, C
Hepadnaviridae	Orthohepadnavirus	Hepatitis B virus
Retroviridae	Human endogenous retrovirus	Human endogenous retrovirus
Anelloviridae	Alphatorquevirus Betatorquevirus Gammatorquevirus	Torque teno virus 5, 8, 11, 16, 24, 29 Torque teno midi virus 1 Torque teno mini virus 5, 6, 7, 8 Unclassified
Parvoviridae	Bocavirus Dependovirus	Human bocavirus 2, 4 Adeno-associated virus 2
Paramyxoviridae	Rubulavirus	Human parainfluenza virus 2
Orthomyxoviridae	Influenza B virus	Influenza B virus
Astroviridae	Mamastrovirus	Human astrovirus 4
Caliciviridae	Norovirus Sapovirus	Norwalk virus Sapporovirus
Hepeviridae	Hepevirus	Hepatitis E virus
Picornaviridae	Enterovirus Kobuvirus Cosavirus Parechovirus Salivirus	Human enterovirus A, B, C, D Human rhinovirus B Aichivirus Human cosavirus Human parechovirus Human salivirus

Technical Appendix Figure 1. Distribution of the percentage of sequence read matches. (A) The 725305 sequence reads were assembled in 78676 contigs and 156008 single reads, which were analyzed using BLASTn searches. The contigs and single reads were classified based on the taxonomic origin in the annotation of best hit sequence (E-score $<10^{-3}$) as being of bacterial, eukaryotic, other or viral origin and depicted in a pie chart colored according to the key. The percentage of each category is based on the number of reads per category. A large percentage of reads showed no hits with any sequence in the Genbank database. (B) Taxonomic classification of sequences with similarity to known viruses. Circles located next to taxa are proportional to the total number of sequence reads identified in the indicated category of viruses with BLASTn ($E<10^{-3}$). The number of sequence reads is included in brackets and the different colors indicate whether the virus infects mammalians, plants, insects or bacteria. The table indicates which virus species were identified.



Technical Appendix Figure 2. Phylogenetic tree of the nucleotide sequences of the partial RdRP sequence of selected representative recoviruses and the newly identified recovirus Bangladesh/289/2007. Phylograms were generated using MEGA5, with the Neighbor-joining method with p-distance and 1,000 bootstrap replicates. Significant bootstrap values and Genbank accession numbers are shown, as well as genogroups and genotypes.

Technical Appendix Table 1. Overview of sequence reads and contigs

No. samples	Read data					No. contigs	Average no. reads/sample
	Total no.	Average length, nt	No. assembled	No. unassembled	% Assembled		
105	725305	222	569297	156008	78.5	78676	6908

Technical Appendix Table 2. Oligonucleotides primers

Primer	Nucleotide sequence, 5′ → 3′	Orientation	Position*
VS431	GCGAGCACAGAATTAATACGACT	Reverse	Not applicable
VS432	CGCGGATCCGAATTAATACGACTCACTATAGG	Reverse	Not applicable
VS627	GGAGGGAAGATGCTG	Forward	527–543
VS628	GACAGGTTCGTCCTTGAG	Reverse	1642–1659
VS629	GCCGCTTTCGCGACTGGTC	Forward	4396–4414
VS630	GACTGGTCTAGGCCAAGCTTC	Forward	4407–4427
VS631	CAATTATACCAAGAAAGATC	Forward	1530–1549
VS634	CCAGAACCAGGAAGAATC	Reverse	4285–4302
VS635	CTATAGTATGTATGGTGG	Forward	4195–4212
VS636	GTGAGCTAAGAGAAGAC	Reverse	4905–4921
VS637	CAGGACCTGAGAGATCTG	Forward	−2 to 16
VS640	CCAGCTGTGTGTATTGC	Reverse	1129–1145
VS664	FAM-CACTGGATTGCCTCAAC-TAMRA	Forward	2077–2093
VS665	CAAGGTGATTGTGAATGACCAACT	Forward	1989–2012
VS666	CGATTGGCCAGTTTACAGC	Reverse	2134–2152

*Position on genome of calicivirus Bangladesh/289/2007 (GenBank accession no. JQ745645).