was not the case in our study. Genetic differences in circulating CHIKV lineages could also explain poor testing performance. Furthermore, the OnSite Chikungunya IgM Combo CE POC test uses a recombinant antigen covering the 226 residues of the E1 gene from CHIKV variant A226; recent studies on CHIKV protein characterization showed that more sensitive serologic assays can be obtained using specific early-phase E2 glycoprotein as antigens (*3*).

The successful use of rapid immunochromatography-based assays with monoclonal antibodies to detect viral diseases (e.g., dengue) has encouraged the development of rapid immunoassays for CHIKV antigens, and preliminary results for these assays seem promising (*6*). External quality assessment programs for POC tests and quality controls consisting of standardized positive serum could also be helpful for improving the performance of diagnostic tests.

In conclusion, returning travelers are sentinels of the rapidly changing epidemiology of CHIKV; thus, they require a prompt diagnosis and careful surveillance for their possible role in subsequent autochthonous disease transmission. Implementation of user-friendly, rapid, and easily deliverable POC tests for a prompt and accurate laboratory diagnosis is therefore needed to improve patient management and disease control measures.

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Novel Single-Stranded DNA Circular Viruses in Pericardial Fluid of Patient with Recurrent Pericarditis

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To the Editor: Circular replication initiation protein (Rep)–encoding single-stranded DNA (ssDNA) (CRESS-DNA) genomes are found in diverse group II virus families, which all possess a conserved Rep-encoding gene and a nonenveloped icosahedral capsid, except gemini-viruses, which have twinned particles (*1*). Gemycircularvirus (GcV) were initially discovered in fungi, but a growing number of new species has been characterized by metagenomics in air, sewage, insects, and feces from a broad range of vertebrates (*1–5*). GcVs have also been found in the brain and serum of humans with multiple sclerosis; in the cerebrospinal fluid of a patient with encephalitis; and in several blood samples, including those from an HIV-positive blood donor (*6–8*). We report the presence of 2 divergent GcVs and a novel CRESS-DNA virus (CV) in 2 pericardial fluid samples from a patient with idiopathic recurrent pericarditis.

The patient, a 14-year-old girl who had thoracic sco-liosis surgery in 2007, was admitted to the hospital in 2009 for pleuropneumonia and pericarditis, which required peri-cardial drainage twice within 3 weeks (samples PF_1 and PF_2, respectively). She had thrombocytopenia, a leukocyte count within the normal range, and a high C-reactive protein level. Biochemical and cytologic testing, bacterial cultures, and PCR of pericardial fluid samples for cytomegalovirus, varicella zoster and herpes simplex viruses, parvovirus B19, fungal 18S rRNA, bacterial 16S rRNA, and *Myco-bacterium tuberculosis* were negative. Upon approval from the Institut Fédératif de Recherche IFR48 Ethics Commit-tee (Marseille, France) and written informed consent from the patient's parents, we submitted the drainage samples for further investigation.

Virus particles in 0.45-μm filtrates were purified and analyzed by metagenomics as described (9); resulting con-tigs were aligned against the National Center for Biotech-nology Information nonredundant protein database using blastx (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Three con-tigs were of viral origin (viral first hit, E-value ≤1E-03), all belonging to the ssDNA circular viruses. We obtained com-plete genomic sequences by PCR with ad hoc primer pairs and Sanger sequencing technology (online Technical Ap-pendix Table, http://wwwnc.cdc.gov/EID/article/22/10/16-0052-Techapp1.pdf).

We annotated genomes using GeneMark (heuris-tic parameters; http://exon.gatech.edu/GeneMark/) and EMBOSS palindrome (http://emboss.bioinformatics.nl/cgi-bin/emboss/palindrome). Analysis of PF1 enabled characterization of HV-GcV1 (GenBank accession no. KU343136) (Figure). This genome displayed a typical GcV architecture, with a 2,264-nt circular DNA molecule carrying a capsid gene on 1 strand and 2 genes on the op-posite strand, which coded for Rep1 (involved in replica-tion initiation) and Rep2 (involved in replication termina-tion), respectively. A putative hairpin structure showed a nanonucleotide motif, which was thought to be the Rep target, TAATGTTAT. A fourth gene with no homologs in databases was predicted upstream of the capsid gene. Phylogenetic inference from concatenated Rep placed this virus close to another GcV (found in sewage) in a clade comprising 2 other human-associated GcVs (online Tech-nical Appendix Figure 1).

PF_2 contained 2 other viruses: HV-GcV2 (GenBank ac-cession no. KU343137), another GcV, and HV-CV1 (Gen-Bank accession no. KU343138), a novel CRESS-DNA vi-rus. HV-GcV2 (2,262 nt) shares the same stem-loop motif and genomic structure with HV-GcV1, with the exception of the *rep* gene, which is coded by a single open reading frame. HV-GcV2 proteins share low sequence similarity with HV-GcV1 proteins (33% for capsid and 46% for Rep, as determined by blastp [https://blast.ncbi.nlm.nih.gov/

Blast.cgi]). HV-GcV2 belongs to another clade of the phy-logenetic tree that also contains sewage- and bird feces–as-sociated viruses (online Technical Appendix Figure 1). HV-CV1 (2,951 nt) possesses characteristics of CRESS-DNA genomes. Phylogenetic analysis of REP sequences showed
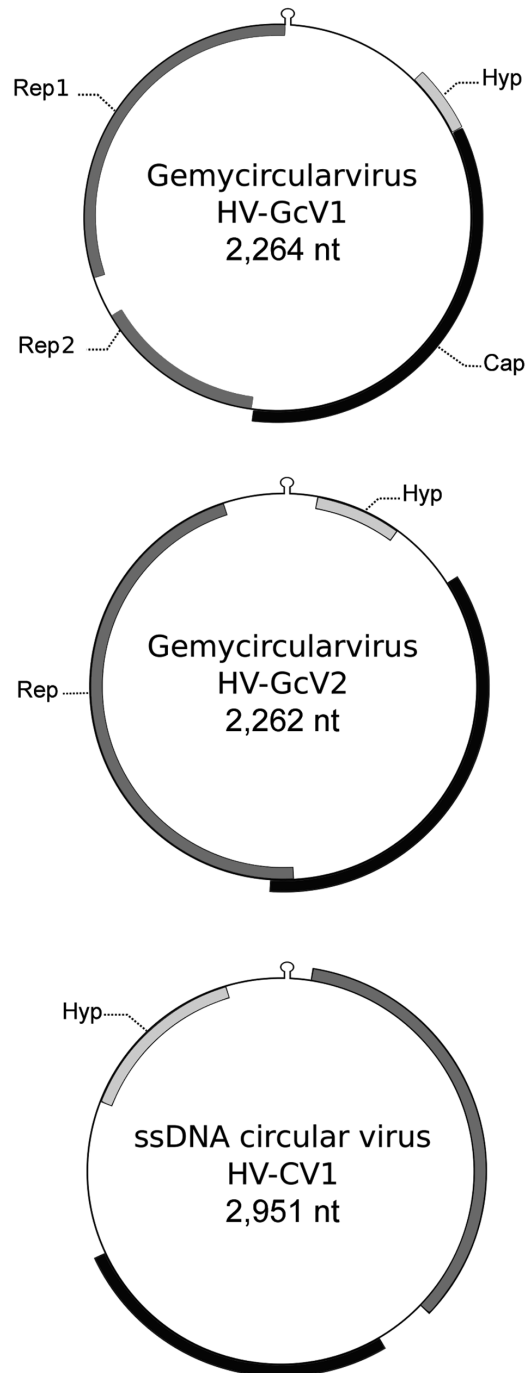

Figure. Genomic features of gemycircularviruses HV-GcV1 and HV-GcV2 and of a novel circular single-stranded DNA (ssDNA) virus, HV-CV1, including hairpin structure and predicted open reading frames. Cap, capsid; Hyp, hypothetical protein with unknown function; Rep, replication initiation protein.

that HV-CV1 and its closest homologue, an ssDNA circular virus of unknown taxon discovered in an Antarctic shelf pond, are distantly related to other CRESS-DNA viruses (online Technical Appendix Figure 2). HV-GcV2 and HV-CV1 displayed no capsid protein similarity between them or with any other virus, as determined by blastp. Annotation of the HV-CV1 capsid gene required use of HHBlits (https://toolkit.tuebingen.mpg.de/hhblits), a more sensitive algorithm (E-value 1.2E-06, probability of 97.2%).

PCR confirmed the absence of HV-GcV1 in PF_2 and HV-GcV2 and HV-CV1 in PF_1, suggesting multiple infections before each pericarditis event or a rapid fluctuation in the load of all 3 persisting viruses. An additional blastx search on 53 other virus metagenomes sequenced from pericardial fluids after pericarditis events failed to retrieve these sequences. To exclude the possibility of sample contamination during procedures, we simultaneously treated a sample with the same reagents and kits used for PF_1 and PF_2 and surveyed it by PCR; results were negative. All metagenomes are publically available in the METAVIR (http://metavir-meb.univ-bpclermont.fr) directory under the pericardial fluids heading.

No relationship between these viruses and pericarditis was established. However, the fact that some CRESS-DNA viruses are animal pathogens (*10*) and the growing number of GcVs found in human samples in pathologic contexts (*6,7*) indicate that the viral genomes described here might replicate in human cells, possibly as opportunistic pathogens (*8*). On the other hand, although diagnostic tests ruled out fungal or bacterial infections, we should still consider the possibility that these viruses infect other uncharacterized organisms. The genomes described here will assist further studies of the prevalence of these viruses in human populations.

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Reemergence of Japanese Encephalitis in South Korea, 2010–2015

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To the Editor: Japanese encephalitis (JE) is caused by a virus transmitted by *Culex tritaeniorhynchus* mosquitoes. JE was the major public health concern in South Korea until the late 1960s, with several thousand cases reported annually. The national vaccination program with the inactivated mouse brain–derived Nakayama strain was initiated in 1983 and targeted children <15 years of age. During 1983–2000, annual booster vaccinations were given to children <15 years of age, but in 2000, the booster schedule was changed to 2 doses (1 dose each) for children 6 and 12 years of age. The live attenuated JE vaccine SA

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# Novel Single-Stranded DNA Circular Viruses in Pericardial Fluid of Patient with Recurrent Pericarditis

Technical Appendix

Technical Appendix Table. PCR primer pairs used to recover whole-genome sequences of gemycircularviruses

Primer pair	Forward primer sequence 5′→3′	Reverse primer sequence 5′→3′
HV-GcV1–1	TTATATGCCCAGACGGACCC	ATTGTGCGGCGGATAGGATA
HV-GcV1–2	CGAATTTAACCCCGGATGCA	AAGGATGCCACCCGAATGTA
HV-GcV1–3	TTGTTCGATCAGACCACCGA	GTTCCTTCCGAGCTACAAGT
HV-GcV1–4	TCGATGTTAACTCCCTCCGG	GAAACGTGTAGATCGGCGAC
HV-GcV2–1	TTGCTCTCAACCGAAAACGG	GCCTGTTACATCGCCGATTT
HV-GcV2–2	TCCGCGTCTTTTGTAGGTCT	AAAGCGTGTGATACCCAAGC
HV-GcV2–3	TCCTTCACCAGCCTCAAGTG	TCTCCGCATGATCAGATCCG
HV-CV1–1	GGAGTTGCATGGGATCGAAC	ACCTTGAGTGACATGAGCCA
HV-CV1–2	TGGGAGTTTGGGGAGATGTC	GAATGTAGTTGGTGCAGGGC
HV-CV1–3	ACGGGCTACACATTCAGCAT	TAGACGCAGAGGATGGATCG
HV-CV1–4	AAGCTTCTTCTCGAACGTCTT	GTTGAAGGACTGGCGGAATC
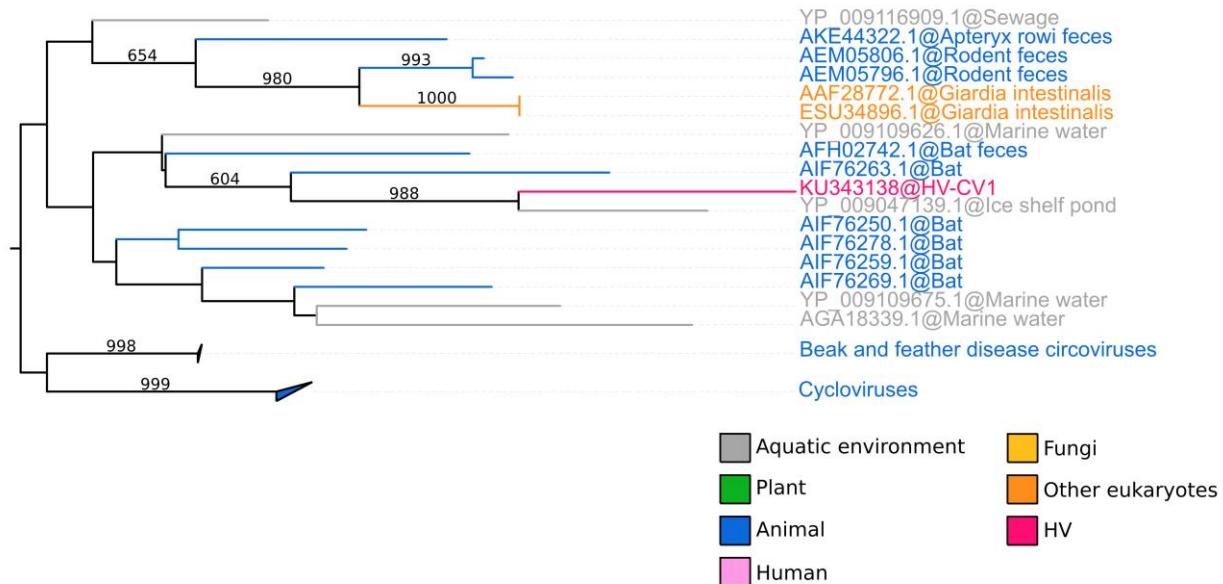
Technical Appendix Figure 1. Maximum likelihood phylogenetic inference of gemycircularvirus replication initiation proteins. Numbers on branches correspond to bootstrap values deduced from 1,000 replicates. Only scores >600 are displayed.
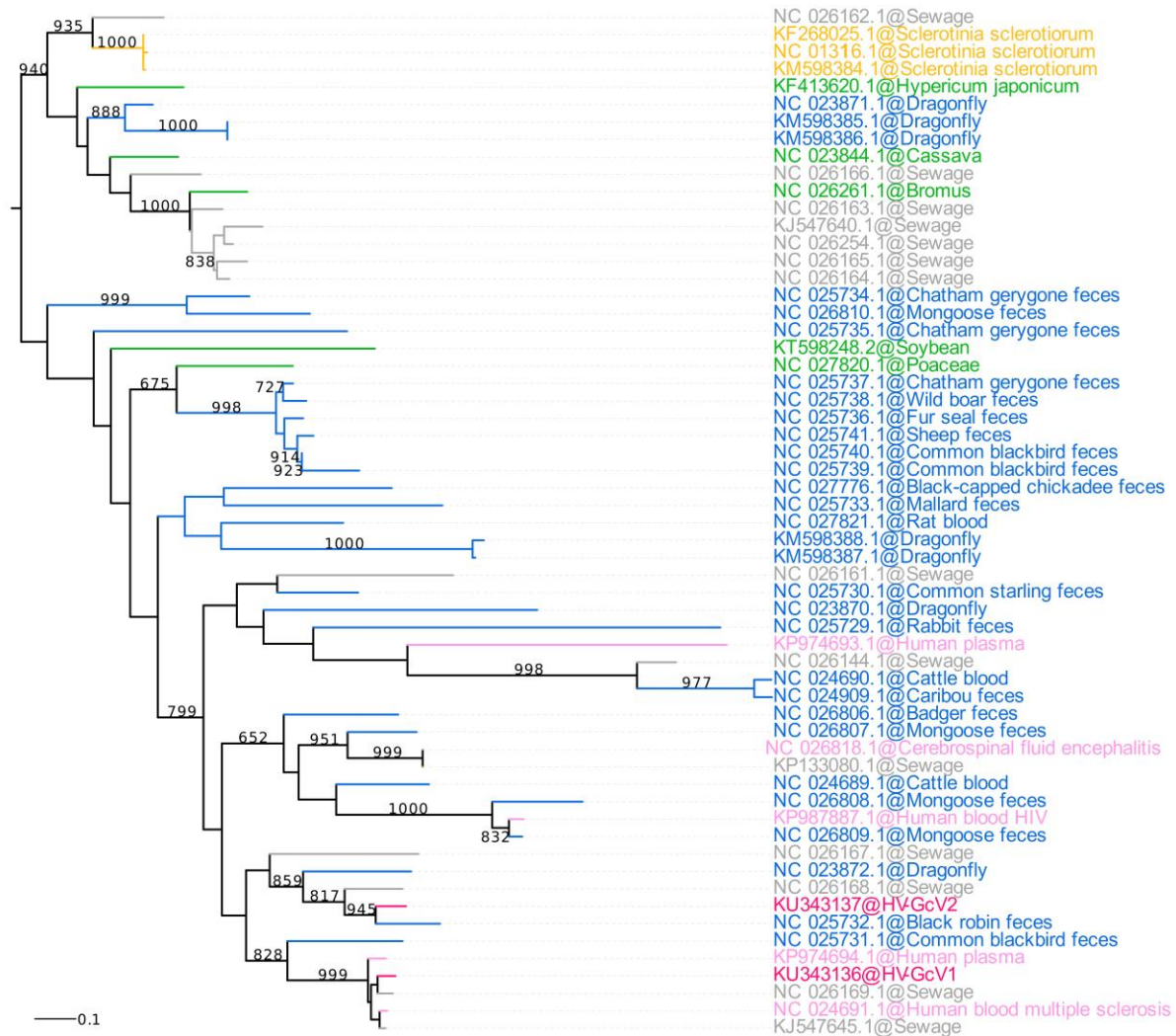
Technical Appendix Figure 2. Maximum likelihood phylogenetic inference of the closest homologues of the HV-CV1 replication initiation protein. Numbers on branches correspond to bootstrap values deduced from 1,000 replicates. Only scores >600 are displayed. Scale bar represents 0.1 residue substitution per site.